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Editorial*

"Minus Minus" Phenotypes

MOST HUMANS have on their red blood cells at least one antigen from each of the first nine blood-group families. This is a universal truth of obvious importance, even though its significance is not now apparent. The simplest possibility is that each of the blood group systems provides essential materials for the construction of the red cell envelope, and that the most satisfactory materials are those produced by the common genes. Occasionally persons are encountered who have no detectable antigen of one of the blood group families: the term "minus minus" has been used by Race and Sanger[13] to describe such phenotypes, as well as other phenotypes in which alternative antigens are lacking though other antigens of the system are still present. These rare types are a source of great interest and speculation, both as to their significance and their genetic explanation.

The original "minus minus" phenotype was Landsteiner's common type O,[9] which was long considered as merely the absence of both the known antigens A and B. Then, in 1927, Schiff[17] discovered that type O bloods all had the antigen H, which was interpreted as indicating that the *O* gene, allelic to *A* and *B*, produced antigen H, and type O seemed neatly explained. The genetic interpretation of common type O had to be reconsidered when Levine, *et al.*,[10] showed that the "Bombay type O" was explainable by the action of genes independent of the *ABO* genes, a milestone in our comprehension of the complexity of blood groups. Common type O does not, however, except for historical reasons, belong in the company of the other "minus minus" phenotypes as these are defined.

Quite recently, many other "minus minus" phenotypes have been found, and a list is shown in Table 1. The list is now almost complete in the sense that examples of the "minus minus" phenotype have been found in each instance where reciprocally related antigens are known, except for M and N. There is no such phenotype in the Wright, Diego, or Sutter systems because no alternatives to antigens Wr^a, Di^a, and Js^a are known. If Wr^b, Di^b, and Js^b are found, it can be confidently predicted that types Wr (a—b—), Di (a—b—) and Js (a—b—) will also eventually be found. The important question, however, is the basic nature of these phenotypes. There appear at present to be several mechanisms by which they can be produced.

Four general classifications are shown in Table 1. Type #1, the common type O, has been mentioned. Type #2, the Bombay type, was shown by Levine, *et al.*,[10] to be caused by the absence of gene *X*, a gene independent of the *ABO* genes, which nor-

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TABLE 1. *"Minus Minus" Phenotypes*

Classification	System	Phenotype	Discoverer	Explanation
Type #1	ABO	Type O (A–B–)	Landsteiner[9]	?
Type #2	ABO	Bombay type (A–B–H–)	Bhende, *et al.*[4]	modifier gene?
Type #3	Rh	C–c–E–e–D+ (Hr_o–)	Race, *et al.*[12]	"partial deletion"?
	MN	S–s– (U–, *M* produces no N)	Greenwalt, *et al.*[5]	
Type #4	Lewis	Le (a–b–)	Andresen[3]	amorphs?
	Duffy	Fy (a–b–)	Sanger, *et al.*[15]	
	P	P–Tj (a–)	Sanger[16]	
	Kell	K–k–Kp ((a–b–) (Ku–)	Chown, *et al.*[6]	
	Kidd	Jk (a–b–)	Pinkerton, *et al.*[11]	
	Rh	C–c–E–e–D–	Vos, *et al.*[18]	
	Lutheran	Lu (a–b–)	Crawford, *et al.*[7]	

mally produces H substance or an ABH precursor. Common type O is therefore, possibly, a combined effect of gene *X* and the absence of any *ABO* gene. Gene *X* thus is visualized as producing the basic ABO substance, which may then be modified by *A* or *B* genes to produce types other than O.

The nature of type #3 (Table 1) is even less well understood. In these phenotypes there is absence of some, but not all, of the antigens known to belong to the particular system. The type –D– (C–c–D+E–e–) [12] was originally considered to be caused by a deletion of *C* genes and *E* genes. This is still an acceptable possibility. Several other, generally similar, varieties of Rh "minus minus" phenotypes have been reported. In the MN system, the type S–s–[8] is comparable. As with the common type O, it is possible that still undiscovered alternatives to C, E, or S may turn up, but one fact argues against this happening. In all these cases, other "expected antigens" (in my opinion) also are missing. Type –D– lacks (in addition to C, cE, and e) the antigen Hr_o, as do also the other "minus minus" phenotypes of the Rh system. Type S–s– lacks also the U antigen; furthermore, the gene that produces M but no S or s also fails to produce any N, whereas other *M* genes do produce some N antigen.[1] As Race and Sanger pointed out,[13] another possible explanation of these phenotypes is a small inversion, or translocation, the inverted or misplaced piece, in unfamiliar surroundings, not behaving normally.

In the fourth category of Table 1 are seven types in which the individual may be homozygous for an amorphic (do-nothing) gene. In the Lewis system this amorph would be the gene *le* of Ceppellini,[5] which fails to produce any identifiable Lewis substance. In the Duffy, Kidd, and Lutheran systems, the "minus minus" phenotypes are tentatively assumed to be due, or possibly due, to homozygosity for the amorphic genes *Fy*, *Jk*, and *Lu*. The P system is not strictly comparable to the others, because P and Tj^a have not been proven to be alternative characters; nevertheless, the "minus minus" phenotype is considered to occur in persons homozygous for the amorph *p*.[16] The Kell type K–k–Kp (a–b–) of Chown, *et al.*,[6] is known to produce also no Ku,[2] and there is unpublished evidence of still other Kell antigens not produced by this Kell amorph, which is presently known as K^o. The amorph that produces none of the Rh factors is known as – – –.[18] Any of the types in category 4 may eventually be shown to be genetic deletions, inversions, or translocations. Or, possibly, other antigenic alternatives, such as H in the ABO system, may turn up. None of these types appears to be caused by independent modifier genes, on the basis of family studies that have been reported. The high frequency of type Fy (a–b–) in Negroes (the one known exception to the general rarity of "minus minus" types), suggests that this may be different from the others of category 4, Table 1. It may, for example, more closely resemble the common type O.

In any or all of the known "minus minus" types, there may be present still another antigen of the same blood-group family, which is so universal that it cannot be detected as a blood-group factor. Conceivably, such universal antigens might be detectable by dosage titrations of serum or red cell eluates from persons with idiopathic acquired hemolytic anemia. I have no idea how much work may have been done along this line; such investigations would be based on the assumption that auto-antibodies might be formed that would detect such antigens, just as other auto-antibodies have detected the antigen e and others, and also that the "minus minus" phenotype might have an excess of the universal antigen. It seems unlikely that many, if any, of the presently-known "minus minus" types are homozygous for a gene that produces an uncommon antigen; most of the large number of rare antigens have been quite thoroughly investigated in exploration of this possibility. In not a single case* has an uncommon antigen been found to fill the gap caused by absence of two alternative antigens, a quite astonishing fact considering the large numbers involved.

Most of the "minus minus" phenotypes are very rare. This is consistent with the idea that the "amorphs" are simply genes lost by deletion. Presumably deletions, even small ones, would be disadvantageous in terms of genetic "fitness," which would keep the frequency of such chromosomes very low. The very high incidence of consanguinity in known examples of "minus minus" phenotype in the Kell and Rh systems (Race and Sanger[14]) is an interesting and unexplained facet of the problem.

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* Except for the debatable case of C^w in the phenotype C—c—C^w+D+E—e—.

The Expected "Bombay" Groups Oh^{A_1} and Oh^{A_2}

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An American family of English extraction is reported in which two Oh ("Bombay") members have transmitted A_2 genes to their children thus demonstrating that the expression of the gene A_2, like that of the gene *B*, can be suppressed in this phenotype. Another American family, of French extraction, shows with a high degree of probability that the expression of the gene A_1 can be similarly suppressed.

THE "Bombay" phenotype was described in 1952.[2] The red cells and saliva of people belonging to this phenotype lack demonstrable A, B and H antigens and their serum contains anti-A, anti-B and anti-H.

In 1955 Levine, Robinson, Celano, Briggs and Falkinburg described a family in which the "Bombay" phenotype was associated with suppression of the action of the gene *B* and of the secretor gene *Se*; the formation of the antigen H was also suppressed. The suppression was clearly genetic and was attributed to genes which were named *Xx*: *X* being very common and *x* very rare in the general population. Suppression reflected the presence of two *x* genes. The convenient symbol Oh was proposed for the "Bombay" phenotype.

It was of interest to know whether Oh could be the manifestation also of the suppression of the action of an *A* gene; but for five years the only families reported[1, 10, 11] did not give evidence for or against suppression of A or B. The second and third families in which suppression was proved to be behind the Oh phenotype were investigated by Hakim, Vyas, Sanghvi and Bhatia (1961). In one of these families it is again the antigen B which is suppressed: in the other the suppression is either of B or of A or of both, but the family groups do not disclose which.

Two Oh families are here reported: the demonstration of the suppression of A_1 in the Pr. family depends on arguments of probability, but in the Be. family the suppression of A_2 is obvious.

The family Pr.

The proposita, Mrs. Pr., was a patient in St. Vincent Hospital, Worcester, Massachusetts. She is a Caucasian of French extraction. Her blood was grouped for a transfusion which, in the end, was not needed. The patient is Rh negative; the finding of an Oh, Rh negative prospective donor would have been practically impossible had not one of her brothers also proved to be Oh, Rh negative. Mrs. Pr. has no detectable anti-Rh in her serum, which is not surprising since both her Rh positive children are, of necessity, ABH incompatible.[12]

There is no known consanguinity in this family.

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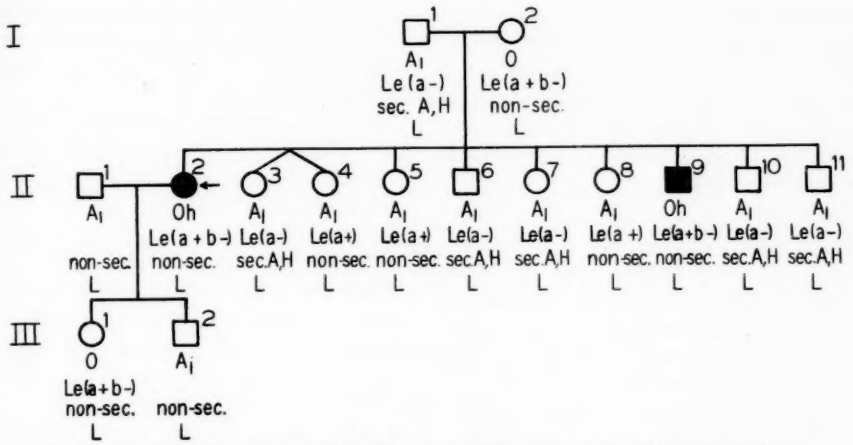

FIG. 1. The Pr. family. Black = Oh, hollow = not Oh; arrow = propositus. Le (a+) etc. = Lewis phenotype of red cells; L = Le^a substance present in saliva.

TABLE 1. *The Groups of the Two Families*

	ABO	MNSs	P	Rh	Lu^a	K	Le^a	Le^b	Fy^a	Fy^b	Jk^a	Jk^b	saliva Secretor	saliva Le^a	saliva Le^b
Pr. family															
I-1	A_1	*Ns/Ns*	P_1	*rr*	—	—	—		—				sec.	+	+
-2	O	*MS/MS*	P_2	*rr*	—	—	+	—	—				non-sec.	+	
II-1	A_1	*Ms/Ns*	P_2	R_1R_2	—								non-sec.	+	
-2	Oh	*MS/Ns*	P_2	*rr*	—	—	+	—	—				non-sec.	+	—
-3	A_1	*MS/Ns*	P_1	*rr*	—	—	—		—				sec.	+	+
-4	A_1	*MS/Ns*	P_2	*rr*	—	—	+		—	+			non-sec.	+	—
-5	A_1	*MS/Ns*	P_1	*rr*	—	—	+		—				non-sec.	+	—
-6	A_1	*MS/Ns*	P_2	*rr*	—	—	—		—	+			sec.	+	+
-7	A_1	*MS/Ns*	P_1	*rr*	—	—	—		—	+	+	+	sec.	+	+
-8	A_1	*MS/Ns*	P_1	*rr*	—	—	+		—	+			non-sec.	+	
-9	Oh	*MS/Ns*	P_2	*rr*	—	—	+	—	—	+			non-sec.	+	—
-10	A_1	*MS/Ns*	P_1	*rr*	—	—	—		—	+			sec.	+	+
-11	A_1	*MS/Ns*	P_2	*rr*	—	—	—		—	+			sec.	+	+
III-1	O	*Ns/Ns*	P_2	R_1r	—	—	+	—	—				non-sec.	+	
-2	A_1	*MS/Ns*	P_2	R_2r	—								non-sec.	+	
Be. family															
II-1	Oh	*Ms/Ns*	P_1	R_1r	—	—	+	—	+	+	+	+	non-sec.	+	—
-2	O	*Ms/Ms*	P_2	R_2r	—	—	—	+	+	+			sec.	+	+
-3	A														
-4	Oh	*Ms/Ns*	P_1	R_2r	—	—	+	—	+		+	+	non-sec.	+	—
-5	O	*Ns/Ns*	P_1	R_2R_2	—	—	—	+	—	+					
-6	B	*MS/Ms*	P_1	R_2r	—	—	—		—	+	+	+			
-7	A_2	*Ms/Ns*	P_1	R_2r	—	—	+	—	+	+	+	+			
III-1	A_2	*Ms/Ms*	P_1	*rr*	—	—	+	—	+	—			non-sec.	+	—
-2	A_2	*Ms/Ns*	P_1	*rr*	—	—	—	+	+	+			sec.	+	+
-3	A_2	*Ms/Ns*	P_1	R_1r	—	—	—	+	+						
-4	A_2	*Ms/Ns*	P_2	R_2r	—	—	—	+	—	+	+	+	sec.	+	+
-5	A_2B			*rr*											
-6	A_2	*Ms/Ns*	P_1	R_2r	—	—	+	—	—	+	+	+			

Antisera used: anti-A, -A_1, -B, -A+B, -H, -O; anti-M, -N, -S, -s; anti-P_1; anti-D, -C, -c, -C^w, -E, -e; anti-Lu^a; anti-K; anti-Le^a and, in the absence of A_1, anti-Le^b; anti-Fy^a, and sometimes anti-Fy^b, anti-Jk^a, -Jk^b. Pr. II-2 gave normal reactions with anti-I (positive) and anti-i (negative). The amount of H in the saliva of Pr. II-3 and II-7 was considerably less than that in the saliva of other A_1 members of the family.

The family Be.

The propositus, Mr. Be., a patient of English extraction at the University of California Medical Center was brought to notice because of difficulty in crossmatching prior to prostatectomy for benign prostatic hypertrophy.

The parents of the propositus were first cousins.

Results

The pertinent groups of the two families are shown in the pedigrees (Fig. 1, 2): fuller groups are given in Table 1. Each propositus has one Oh sib.

Identification with Oh

The identification of the phenotypes of Pr. II-2 and II-9 and Be. II-1 and II-4 with previous examples of Oh depends on the following tests: (1) The sera of the four people agglutinated all red cells tested except their own, each others and those from two Oh persons, Donor Z[2] and Mr. P.K.R.[10] Furthermore, the agglutination could be inhibited by secretor saliva. (2) The cells of the four people were not agglutinated by the serum of the following Oh persons: the original "X," "Y" and "Z,"[2] Mr. P.K.R.,[10] X.H.M.[13] and Mr. M.[7]

Saline suspensions of the cells of Pr. II-2 and II-9 were not agglutinated by anti-A (including "immune" examples), anti A_1 (human and *Dolichos*), anti-B (including one "immune" example), anti-A+B, anti-H (human and *Ulex*) nor by "anti O" (human). The immune anti-A and anti-B did not react with the cells of II-2 or II-9 when Löw's papain method was used. No anti-A could be eluted from the cells after exposure to immune anti-A.

The cells of Be. II-1 and II-4 were less exhaustively tested: they were negative with anti-A, anti-A_1 (human and *Dolichos*), anti-B, anti-A+B and anti-H (human and *Ulex*).

(3) Like other examples of Oh, Pr. II-2 and II-9 and Be. II-1 and II-4 are nonsecretors of A, B and H substances and their saliva contains Le^a substance (but not Le^b substance).

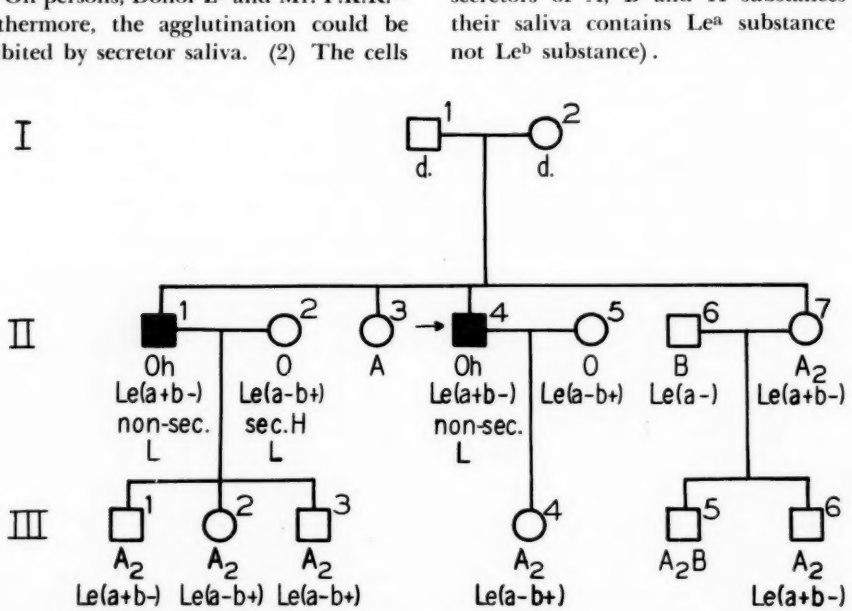

FIG. 2. The Be. family. I-1 and I-2 are first cousins.

TABLE 2. *Inhibition and Absorption Tests on the Serum of Four Oh People*

	Serum / Cells	Pr. II-2 O	Pr. II-2 A_1	Pr. II-2 A_2	Pr. II-2 B	Pr. II-9 O	Pr. II-9 A_1	Pr. II-9 A_2	Pr. II-9 B	Be. II-1 O	Be. II-1 A_1	Be. II-1 A_2	Be. II-1 B	Be. II-4 O	Be. II-4 A_1	Be. II-4 A_2	Be. II-4 B
1	serum + saline	4	4	4	4					4	4	4	4				
2	" + O non-scc. saliva	4	4	4	4	2	2	1	3	3	4	3	4	1	3	1	3
3	" + O sec. saliva	–	4	1	4	–	1	–	3	–	3	–	3	–	3	w	3
4	" + A sec. saliva	–	1	–	4	–	–	–	3	–	–	–	2	–	–	–	3
5	" + B sec. saliva	–	4	w	–	–	w	–	–	1	3	2	–	–	2	–	–
6	" + A subst. (Knickerbocker)	–	–	–	4												
7	" absorbed by O cells	–	4	–	4												
8	" " A_1 "	–	–	–	4												
9	" " A_2 "	–	–	–	4												
10	" " B "	–	–	–	–												

In the inhibition tests equal volumes of saliva, diluted 1 in 2 in saline, were added to the serum. In the absorption tests equal volumes of serum and packed cells were used. The agglutination tests were incubated at about 22°C. Scale of agglutination: 4, 3, 2, 1, w, - .

Inhibition and absorption tests

Table 2 summarizes the results of inhibition and absorption tests on the sera of the four Oh people. Anti-B is easily demonstrated in all four sera (rows 4 and 5 and, in Pr. II-2 only, rows 6, 8 and 9); and so is anti-H (it is inhibited by all the samples of secretor saliva).

The presence of anti-A_1 is demonstrated in Pr. II-2 and Be. II-4 (row 5). There is no clear demonstration in Table 2 of anti-A in any of the sera, but when O secretor saliva was added, drop by drop, to the serum of Pr. II-2 until it no longer reacted with O cells then anti-A_1, anti-A and anti-B could all be detected. When the serum of Pr. II-2 is absorbed by B cells (row 10) the reaction for A cells disappears: B secretor saliva does not have this effect.

Other tests

Temperature affects the reactions of the antibodies in the serum of all four Oh individuals; they react more strongly at lower temperatures, as Table 3 shows: in this they differ from the antibodies in the serum of some Oh people[1, 2, 10, 13] but not of others.[9, 14] At 37 C. three of the four sera reacted most strongly with B cells.

For a reason which will be given in the discussion the cells of the two Oh members of the Be. family were tested against titrations of a horse anti-type XIV pneumococcus serum. Their agglutination scores were no higher than those of control O Le (a–b+) and O Le (a+b–) cells, but they were higher than the scores achieved by one B and two A_2 members of the Be. family. After two absorptions with O Le (a–b+) or O Le (a+b–) cells the anti-type XIV serum still agglutinated a sample of Oh cells, rather weakly, but it did not agglutinate two control O samples.

The antibodies in the sera of Be. II-1 and II-4 were localized in the γ globulin fraction by starch block preparative electrophoresis.

The sera of Pr. II-2 and II-9 agglutinated O, A and B cord cells (thus behaving like other Oh sera tested at the Blood Group Research Unit).

The sera of the relatives of the Oh persons in the two families were conventional in their anti-A and anti-B content. This was shown by agglutination tests and confirmed by inhibition tests with saliva from A, B and O secretors.

Discussion

The Be. family (Fig. 2) demonstrates clearly that the "Bombay" phenotype can be the consequence of suppression of A_2 just as it can of B. The propositus, II-4, who is Oh, and whose wife is O, has an A_2

daughter. The elder brother, II-1, is also Oh and his wife is O and they have three A_2 children. The group of II-4 and II-1 may, therefore, be written Oh^{A_2}; the superscript being added, as suggested by Dr. Levine, after family tests have shown which gene has been carried but prevented from expressing itself as an antigen.

TABLE 3. *The Effect of Temperature on the Scores Given by Various Red Cells When Tested Against Titrations of the Serum of the Four Oh Persons*

	Cells			
	O	A_1	A_2	B
Pr. II-2 serum, 37° C.	8	6	10	17
" " 22° C.	27	41	30	37
" " 8° C.	37	53	37	47
Pr. II-9 serum, 37° C.	2	2	0	20
" " 22° C.	10	13	9	35
" " 8° C.	18	25	18	41
Be. II-1 serum, 37° C.	27	37	29	32
" " 22° C.	32	40	29	32
" " 8° C.	37	49	34	41
Be. II-4 serum, 37° C.	13	15	13	27
" " 22° C.				
" " 8° C.	30	31	25	35

In the Pr. family the demonstration that A_1 has been suppressed is less straightforward because II-2 cannot be proved to have transmitted an A_1 gene to the next generation. The argument that she has an unexpressed A_1 gene depends on the genotype of her father. The father, I-1, has given an A_1 gene to all the eight of his children who are not of the Oh type. He is, therefore, probably of the genotype A_1A_1: the chance of his being A_1O is only $(\frac{1}{2})^8$ or 1 in 256. If he were A_1O the chance that both his Oh children would lack A_1 is 1 in 4. The total chance therefore that neither of the Oh people has A_1 is only 1 in (256×4) or 1 in 1,024.

We know that *xx* inhibits the formation of B and of H antigen: it seems reasonable to suppose that the same *xx* genes are responsible for the inhibition of A and of H antigens in the present two families. Such a conclusion agrees with the theory developed by Watkins and Morgan[15, 16] and by Ceppellini[4] that the genes *A* and *B* convert a precursor mucopolysaccharide H more or less completely to their own stamp, and that the Oh phenotype results from the absence of a gene *H* necessary for the production of this precursor H substance. Should this very attractive theory ultimately be substantiated then the suppressor genes *X* and *x* could more appropriately be called *H* and *h*—a notation suggested by Watkins and Morgan[16] when considering the "Bombay" phenomenon before the finding of the first family that demonstrated the suppression of B.

According to Watkins and Morgan[17] the only serological activity of ABO and Lea precursor substance so far demonstrated is the capacity to cross-react with anti-type XIV pneumococcus serum. According to the current theory Oh cells should contain unmodified ABH precursor substance. It was therefore disappointing to find that Oh cells reacted no more strongly than O cells with the anti-type XIV serum. Perhaps the theoretically possible Oh cells which are Le (a—) will, if ever found, give a different result.

Ceppellini,[5] the first to foresee suppression as an explanation of Oh, considered that the suppressor gene might belong to the Lewis system.

If we assume that the *Xx* genes are responsible for the Oh phenotype in the four families so far published[1, 9, 10, 11] and for the two reported in this paper, then some negative information is available about the location of these genes.

In the family Be., I-1 and I-2 are first cousins; therefore they have identical copies of the same *x* gene (since we may reasonably assume that it has come to each from the same grandparent). It follows that the *Xx* genes cannot belong to any locus for which either of the Oh sibs are heterozygous. Thus the Be. family shows that the *Xx* genes do not belong to the MNSs, P, Rh, Duffy or Kidd loci.

In families with or without consanguin-

ity another argument can be used. If the *Xx* genes belonged, say, to the Rh locus (which they do not) then *xx* sibs would have to have the same Rh groups. It follows that if *xx* sibs differ from each other on any system then the *Xx* genes do not belong to that system.

We have applied these two methods to the four Oh pedigrees previously published, and the *Xx* genes are shown not to belong to the following loci: ABO, Secretor,[9] MNSs,[10] P,[11] Rh,[1, 10, 11] and Duffy.[1] The *Xx* genes are not sex-linked: if they were the son of Pr. II-2 would be Oh, but he is not.

The *Xx* genes do not belong, therefore, to any of the following loci: ABO, MNSs, P, Rh, Duffy, Kidd or Secretor; nor are they sex-linked.

The sera of Oh people deserve further investigation: they do not contain simple mixtures of anti-A, anti-B and anti-H though all these antibodies can be identified in them. The problem is likely to be difficult, even more so than that of the specificity of the antibodies in group O sera.[3, 6]

Acknowledgments

We are indebted to Dr. Walter Casale, Director of the Clinical Laboratories, St. Vincent Hospital, Miss Rosemarie Hurley and Miss Mary Armstrong, Technicians, Blood Bank, St. Vincent Hospital, for the clinical assistance needed for this investigation.

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Eleven Cases of "Bombay" Phenotype in Six Families: Suppression of ABO Antigen Demonstrated in Two Families

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Eleven cases of "Bombay" phenotype in six different Indian families are reported. Two of the families show evidence for the suppression of the ABO antigens. In one family B antigen was involved in two sibs with "Bombay" phenotype and in the other suppression was evident but the true ABO groups could not be ascertained. Most cases are identified due to crossmatching difficulty but a few cases are reported in this article which were detected through the cells. The common occurence of these cases from Bombay is pointed out and the importance of using anti-H as one of the reagents in doing ABO grouping in India is stressed.

Bhende, Deshpande, Bhatia, Sanger, Race, Morgan and Watkins[4] in 1952 discovered a new ABO blood group now popularly known as "Bombay": the serum of people of this group contains anti-H as well as anti-A and anti-B and the cells fail to be agglutinated by anti-A, -A_1, -B, -H and anti-O. Three examples were reported and all were Le (a+b–) and non-secretors of ABH antigens. Since then a few more cases have been reported from India,[3, 8, 9] one Irish family with two cases by Parkin[7] and an American family of Italian origin with three cases by Levine, Robinson, Celano, Briggs and Falkinburg.[6]

Originally this blood group was thought to be due to a new allele at the A_1A_2BO locus. Levine, *et al.*,[6] while investigating their family were able to show that there was no new gene at the A_1A_2BO locus, but the ordinary ABO antigens were suppressed on the red cells and in the saliva by the presence of a rare suppressor gene *x* in homozygous condition.

In the past few years we have encountered 11 cases of this character in six different families. Details regarding their caste, community, sex, consanguinity and mode of ascertainment were recently reported by Bhatia and Sanghvi,[2] who found three cases in their series of 11 had consanguinity among their parents.

All these cases have been investigated in detail: red cells were tested with 8 to 10 anti-H sera and the sera were tested with at least two "Bombay" cell samples. Table 1 gives the blood groups of all the members investigated in these families and Table 2 gives the results of titrating the sera of these 11 people against O, A_1, A_2, B, A_1B, A_2B as well as cord O and papainized group O cells at room temperature (approx. 28 C.). Titers in all these 11 cases were the same when the tests were done at different temperatures. All these sera contained anti-A, anti-A_1, anti-B besides anti-H. Absorption with Le (a+b–) and Le (a–b+) did not reveal Lewis antibody in any serum. Cells in many of the cases were also used for absorption and elution tests with several immune anti-A+B (group O) sera and no anti-A or anti-B was eluted. The anti-H in all these cases was inhibited by secretor saliva and meconium but not by non-secretor saliva and meconium. Details of the cases are given below.

Family R. A.: This was an isolated case investigated as above but family investigations could not be done.

Family S. S. B.: Tests on the few available members did not reveal any suppression. The patient and her husband were both non-secretors so there was good opportunity of detecting the suppression of the secretor gene by finding at least one secretor child. However, all her three children were non-secretors.

Family Jak.: Three generations were tested: three more cases of "Bombay" char-

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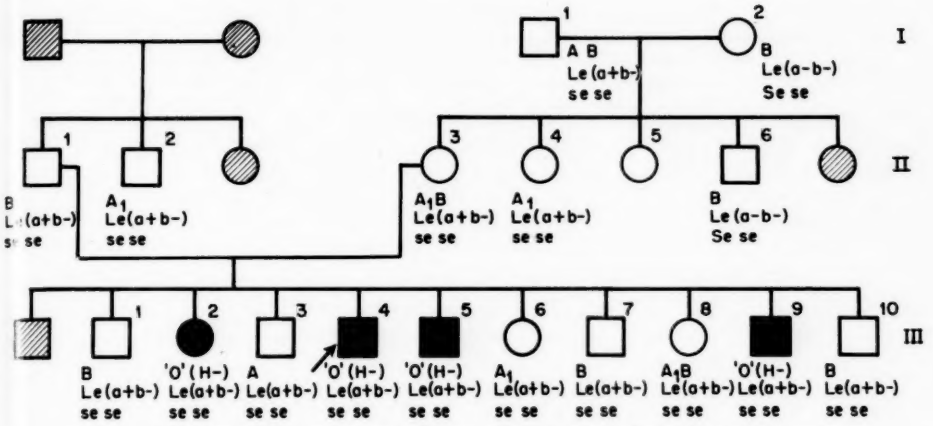

FIG. 1. ABO, Lewis blood groups and Secretor status of the members of the "Jak" family
'O' (H–) = "Bombay" Phenotype. Hatching = Dead.

acter were found amongst the sibs of the propositus (Fig. 1). In this family the serological data appear, superficially, to violate the Mendelian laws of inheritance. The propositus and three of his sibs are apparently group O, but with anti-H in their sera, and their mother is group A_1B: the mating A_1B mother and B father should give rise only to A_1, B and A_1B children and all these groups were found in the remaining sibs of the propositus. The true groups of III-2, III-4, III-5 and III-9 may be A,B or AB. Both the parents were Le (a+b–) and non-secretors of ABH antigens in saliva. The Lewis blood groups therefore do not contribute to the question of suppression of the secretor gene which does not arise in this case, but they do substantiate the genotype of the "Bombay" persons in this family as *sese*.

Family Jip.: This case was identified while testing the cells of a Kokana tribe during an anthropological survey in which all cells were tested with chicken anti-H. The father and two brothers of the propositus were tested: one brother, Mr. S., also had the "Bombay" phenotype. There was nothing informative about the suppression of the ABH antigens.

Family Kr.: Mrs. Kr., aged 30 years, and in her seventh month of pregnancy, came for ante-natal check up. This was her fourth pregnancy; she had three healthy children. During ante-natal Rh testing her

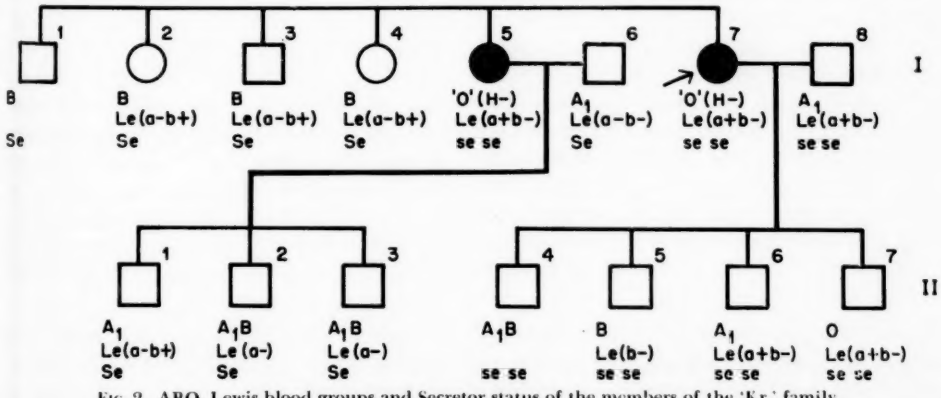

FIG. 2. ABO, Lewis blood groups and Secretor status of the members of the 'Kr.' family.
'O' (H–) = "Bombay" Phenotype.

TABLE 1. *Blood Groups of the Members of 'Bombay' Families*

Family	Name	ABO	Rh	MN	P	Kell	Fy^a	Lewis	Saliva				
									A	B	H	Le^a	$I^{\overline{gb}}$
R.A.	Mr. R.A.	'Bombay'	R_1R_2	MN	P_1			a+b−	−	−	−	+	-
S.S.B.	Mrs. S.S.B.	'Bombay'	R_1R_1	N	P_1		−	a+b−	−	−	−	+	-
	her husband	A_1	R_1r	M	P_2		+	a+b−	−	−	−	+	-
	her son	A_1	R_1r	MN	P_1		−	a+b−	−	−	−	+	-
	her son								−	−	−	+	-
	her daughter								−	−	−	+	-
Jak.	I-1	AB	R_1r	MN				a+b−	−	−	−	+	-
	I-2	B	R_1R_1	MN				a−b−	−	+	+	+	+
	II-1	B	R_1r	M				a+b−	−	−	−	+	-
	II-2	A_1	R_1r	MN	P_1			a+b−	−	−	−	+	-
	II-3	A_1B	R_1R_1	MN				a+b−	−	−	−	+	-
	II-4	A_1	R_1R_1	M				a+b−	−	−	−	+	-
	II-6	B	R_1r	MN				a−b−	−	+	+	+	+
	III-1	B	R_1R_1	MN				a+b−	−	−	−	+	-
	III-2	'Bombay'	R_1R_1	MN	P_2			a+b−	−	−	−	+	-
	III-3	A_1	R_1r	MN	P_1			a+b−	−	−	−	+	-
	III-4	'Bombay'	R_1r	MN	P_1	kk		a+b−	−	−	−	+	-
	III-5	'Bombay'	R_1r	MN	P_1			a+b−	−	−	−	+	-
	III-6	A_1	R_1R_1	MN	P_1			a+b−	−	−	−	+	-
	III-7	B	R_1R_1	M				a+b−	−	−	−	+	-
	III-8	A_1B	R_1r	M				a+b−	−	−	−	+	-
	III-9	'Bombay'	R_1r	MN	P_2			a+b−	−	−	−	+	-
	III-10	B	R_1R_1	MN				a+b−	−	−	−	+	-
Jip.	Mr. Jip	'Bombay'	R_1R_2	N	P_1	kk		a+b−	−	−	−	+	-
	his father	A_1	R_1R_1	N	P_2	kk		a+b−	−	−	−	+	-
	his brother	A_1	R_1R_1	N	P_1	kk		a+b−	−	−	−	+	-
	his brother, S.K.	'Bombay'	R_1R_2	N	P_1	kk		a+b−	−	−	−	+	-
Kr.	I-1	B	R_1r	MN					−	+	+	+	+
	I-2	B	R_1R_1	M				a−b+	−	+	+	+	+
	I-3	B	R_1R_1	M				a−b+	−	+	+	+	+
	I-4	B	R_1R_1	M		kk	+	a−b+	−	+	+	+	+
	I-5	'Bombay'	R_1R_1	MN	P_1	kk	+	a+b−	−	−	−	+	-
	I-6	A_1	R_1r	MN	P_2			a−b−	+	−	+	+	+
	I-7	'Bombay'	R_1R_2	MN	P_1	kk	+	a+b−	−	−	−	+	-
	I-8	A_1	R_1r	MN	P_1	kk	+	a+b−	−	−	−	+	-
	II-1	A_1	R_1R_1	MN	P_1			a−b+	+	−	+	+	+
	II-2	A_1B	R_1r	M				a−	+	+	+	+	+
	II-3	A_1B	R_1R_1	N				a−	+	+	+	+	+
	II-4	A_1B	R_1r	MN		kk	−		−	−	−	+	-
	II-5	B	R_2r	MN		kk	−	b−	−	−	−	+	-
	II-6	A_1	R_2r	MN	P_1	kk	+	a+b−	−	−	−	+	-
	II-7	O	R_1R_1	MN	P_1	kk	+	a+b−	−	−	−	+	-
A.F.	Mrs. A.F.	'Bombay'	rr	N	P_2	kk	−	a+b−	−	−	−	+	-
	her son	O	R_2r	MN	P_1	kk	−	a−b+	−	−	+	+	+

TABLE 2. *Titers of Antibody in the Sera of Eleven 'Bombay' Persons for the Following Red Cells: O, A_1, A_2, B, A_1B, A_2B, Cord O and Papainized O*

Family	Case	O	A_1	A_2	B	A_1B	A_2B	O Cord	O Pap.
R.A.	Mr. R.A.	64	512(2)		512(2)	512(2)		64	256(4)
S.S.B.	Mrs. S.S.B.	512 (4)	128(2)	512(2)	256(2)	256(2)	512 (2)	256	512(4)
Jak.	III-4	128	256	256	128	128	128	64	256
	III-2	128	512	256	512	512	256	128	1,024
	III-5	256	1,024	512	256	512	256	256	1,024
	III-9	1,024	2,048	1,024	2,048	1,024	1,024	512	4,096
Jip.	Mr. Jip.	64	64	64	64	64	64	64	128
	S.K.	32	64	32	32	64	32	32	128
Kr.	I-7	128	128	128	128	128	128	128	256
	I-5	64	128	64	128	128	64	64	128
A.F.	Mrs. A.F.	32	128	64	64	128	64	32	128

Figures in parentheses indicate titer of hemolysins.

blood was tested as a routine with anti-H serum. She delivered a full-term male baby, group O and his cells reacted strongly with anti-H serum. The direct Coombs test on the baby's cells was negative. The cord serum of the baby contained incomplete anti-H (titer 4), anti-A (32) and anti-B (32): though the complete form of the antibody was detectable in small amounts. Eluate from the cord cells had anti-H (titer 2). Saliva, amniotic fluid and meconium of the baby were also tested for the presence of A, B, H and Le^b antigens which were not detected: Le^a antigen was present in all three fluids. There was no rise in the titer of the anti-H in the mother's serum when tested on the tenth day after delivery, and the baby did not have any signs of the hemolytic disease of the newborn.

Results of the family investigations are given in Figure 2. A sister (I-5) is also of the "Bombay" type. These investigations also revealed the suppression of the B antigen in the propositus, I-7, and her sister, I-5. The children of I-7 were group A_1B (II-4), B (II-5), A_1 (II-6) and O (II-7) while the children of I-5 were group A_1 (II-1), and A_1B (II-2, II-3). The finding of group A_1B children to superficially group O mothers is contrary to the law of inheritance of blood group genes and confirms the suppression of antigen B in I-5 and I-7. The presence of group A_1 in the children of I-5 and A_1 and O in the children of I-7 shows that the genotype of I-5 and I-7 must be *BO* and that of the husband, I-8, of the propositus is *AO*. No positive evidence for the suppression of the ABH antigens in saliva could be obtained from the family studies though other sibs of these two sisters were all secretors. This suggests that the secretor gene is very likely suppressed in both I-5 and I-7. If so, two of the children of I-7 should have been secretors. All her children were, however, non-secretors.

Family A. F.: This case was also detected during ante-natal check up. The patient, in N. Wadia Maternity Hospital, had had three normal deliveries. She was found to be Rh negative (*cde/cde*) and to have the "Bombay" character: her serum contained anti-H but no Rh antibody. It was only possible to examine her son's blood and this was group O and reacted strongly with anti-H serum.

Discussion

Interpretation of the findings in the two families, Jak. and Kr., is simplified by the earlier observation of Levine, *et al.*,[6] who reported the suppression of B and H anti-

gens on red cells and in saliva. This confirmed the earlier masterly prediction of Ceppellini[5] who on analyzing the data in the first three "Bombay" cases reported by Bhende, *et al.*,[4] anticipated the possibility of gene interaction of Lewis and ABH secretion system. He also indicated some such mechanism for the red cell antigen. Until recently there was no evidence for the suppression of the antigen A though the chance of one or more of the four "Bombay" persons in the Jak. family being group A or AB could not be excluded. However, suppression of A has now been demonstrated in one family with certainty and in another with a high degree of probability.[1]

The *ABO* genes are transmitted and their effects are fully expressed in the *Xx* individuals who are heterozygous for the suppressor gene. This was the reason for the normal character of the A, B and H antigens in the persons who are the carriers of this rare gene in the present study and also in the earlier cases reported by Bhatia, *et al.*[3]

Such families sometimes appear to violate the laws of inheritance and may thus create a theoretical objection to the use of ABO blood groups in disputed paternity tests. But the objection is not valid for these cases can easily be spotted by the negative reactions of the red cells with anti-H sera and the presence of anti-H in the serum.

The finding of eleven cases in the last few years in six different families suggests that such cases are not very rare in India. An attempt has been made to get the frequency of this phenotype in Bombay. It has been observed that the incidence of the "Bombay" phenotype in the Marathi speaking persons in and around Bombay is of the order of 1 in 13,000 and it can be calculated that 1 in 77 of these people may be carriers of the gene *x* (Bhatia and Sanghvi).[2] Most of these cases were discovered because of difficulty in cross-matching but the few cases like the Donor Z (Bhende, *et al.*)[4] and Mr. Jip., Mrs. Kr. and Mrs. A.F. are the results of a deliberate search by testing cells with anti-H serum as a routine. The finding of the 18 cases in India stresses the importance of the routine use, in India, of anti-H serum for blood grouping.

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Erythrocyte Receptors for Two "Nonspecific" Lectins*

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A comparative study of the inhibition by various substances of (nonspecific) *Bauhinia purpurea* and *Ricinus communis* hemagglutinins reveals many similarities but some definite differences. *Bauhinia* extracts are made N-specific by the presence of D-galactose or of other sugars of Mäkelä's group 2, but *Ricinus* is not. *Bauhinia* is inhibited by some carbohydrates of group 3, and *Ricinus* is not. The erythrocyte receptors with which these two lectins combine are thus apparently not identical. It is suggested that all human erythrocytes contain a considerable number of such receptors, and that only some of the receptors, possibly only the terminal few units of some of them, are altered by the action of genes such as A,B, O; M,N; C, c; etc.

ONE of us[1] has previously reviewed the work of Morgan and Watkins,[9] Krüpe,[7] and Mäkelä[8] which led to the concept that even the "nonspecific" plant agglutinins, or some of them at least, display a certain degree of specificity, in that their agglutinative activity for red cells is inhibited by certain carbohydrates and not inhibited by others. It was suggested also that from the results of such inhibition studies tentative conclusions may be drawn, reasoning as did Watkins and Morgan[10, 11] and Kabat,[6] as to the chemical nature of the receptors in the erythrocytes with which these phytagglutinins combine. We wish to present here the results of a new comparative study of two such nonspecific agglutinins, the one that is found in the seeds of *Ricinus communis*, which was one of the first plant agglutinins to be discovered, and that of *Bauhinia purpurea*, one of the most recent.

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Extracts of *Bauhinia purpurea*, unless certain inhibiting substances such as galactose are present,[2] agglutinate the erythrocytes of all the human beings we have tested, and *Ricinus communis* extracts seem to be nonspecific, in this sense, no matter how they are treated.

Some inhibition studies were made by Krüpe[7] on *Ricinus*, Mäkelä[8] studied *Bauhinia variegata*, and preliminary studies were made on *Bauhinia purpurea* in this laboratory.[2] The present report is based on the results of a new, rather more thorough, investigation carried out on the two lectins simultaneously.

Materials and Methods

The *Bauhinia* seeds were ground fine in a mill and extracted with eight times their weight of 0.9 per cent NaCl solution as described by Boyd, Everhart and McMaster.[2] As there described the lectin was fractionated and redissolved in concentrated solution.

The *Ricinus* seeds were shelled, cut into small pieces, and extracted with a mixture of one part ethanol and two parts ether until apparently fat-free. They were then dried at room temperature, ground in a mortar, and extracted overnight with ten times their weight of 0.9 per cent NaCl solution. The *Ricinus* extract was not further treated, as no treatment tried by us had any effect on its specificity. Suitable dilutions of the clarified *Bauhinia* and *Ricinus* extracts were made with 0.9 per cent NaCl solution.

Our technic of testing for inhibition consisted of mixing in small test tubes one drop of a suitable dilution of the lectin with one

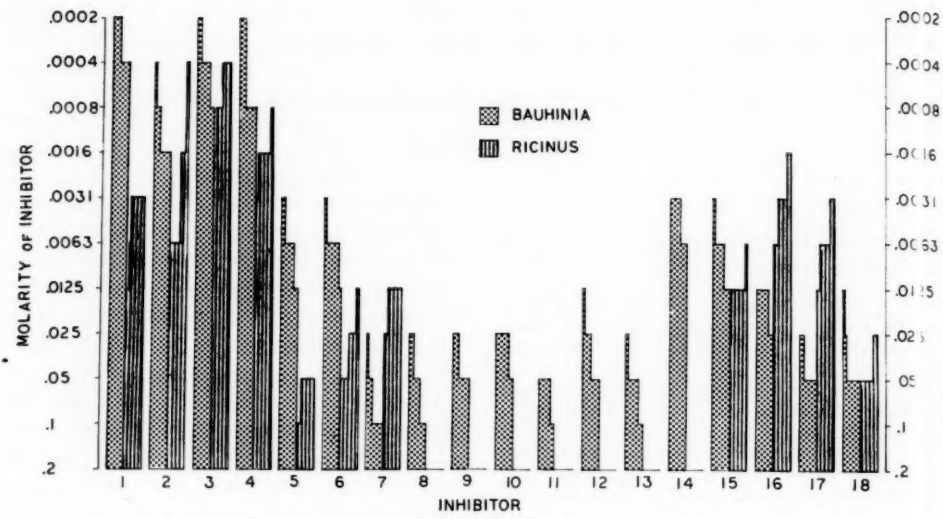

FIG. 1. Inhibition of *Bauhinia* and *Ricinus* extracts by various substances. The width of the vertical columns is proportional to the degree of inhibition at the various concentrations of inhibitor. The substances used are indicated by numbers. They were: **Group (a):** (1) D-galactose, (2) raffinose, (3) lactose, (4) melibiose, (5) D-talose, (6) L-arabinose, (7) D-mannoheptose; **Group (b):** (8) D-erythrose, (9) L-altrose, (10) cellobiose, (11) maltose, (12) turanose, (13) trehalose, (14) salicin; **Group (c):** (15) L-rhamnose, (16) L-mannose, (17) L-xylose, (18) i-inositol. Test erythrocytes as indicated in text.

drop of a suitable dilution of the carbohydrate (and, in the case of *Ricinus,* a drop of 0.25 per cent saline solution of lima bean trypsin inhibitor[4] to prevent the agglutinated red cells from sticking to the walls of the tube), adding a drop of 1 per cent erythrocyte suspension, incubating at 4 C. for an hour, mixing, centrifuging at room temperature, and reading by gentle shaking of the individual tubes over a small mirror.

We generally started with stock 0.2 M solutions of the substances we were studying. These stock solutions were always kept frozen when not in actual use.

Results

A number of the carbohydrates, glycosides and polyols tested had no inhibitory, or very slight inhibitory, effect on either *Bauhinia* or *Ricinus* extracts, even in 0.2 M concentration. These included:

TABLE 1. *Agglutinative Activity of Dialyzed* Bauhinia *Preparations in the Presence of Different Concentrations of a Group 2 Sugar*

Test cells of type	Molarity of Inhibitor (Lactose) Added								
	0.2	0.1	0.05	0.025	0.013	0.006	0.003	0.002	0.001
M	0	0	0	0	0	0	0	0	1
MN	0	0	0	0	0	2.5	2.5	2.5	3
N	0	0	0	0	0	2	3	3.5	4

The numbers in the table indicate the strength of agglutination when erythrocytes were added to the mixture of lectin and inhibitor, the mixture incubated, mixed, centrifuged, and gently shaken (see text). The symbol 4 indicates complete agglutination, 1 weak agglutination, 0 no agglutination.

aconitol	gentiobiose	melezitose
D-allose	D-glucose	D-ribose
D-arabinose	L-glucose	D-sorbitol
L-arabinose	D-gulose	sucrose
dulcitol	D-idose	streptomycin
erythritol	D-lyxose	streptobiosaminide
L-fucose	D-mannose	D-xylose
D-fructose	D-mannitol	L-xylose
L-galactose	D-mannoheptulose	

The sample of stachyose tested by us was hemolytic in the higher concentrations, making it difficult to ascertain whether it inhibited weakly or not.

The results obtained with substances that inhibited one or both of the lectins used in the present study are shown graphically in Figure 1. To facilitate reading off the concentrations of the substances listed toward the right of this figure, the concentrations of inhibitor are shown on a right-hand ordinate also. In order to make the two extracts roughly equal in agglutinating strength, the *Bauhinia* preparation was diluted 1:3 for these studies, and the *Ricinus* extract was diluted 1:1500. Both of the lectins were always tested with M, MN, and N erythrocytes; only the reactions with the M erythrocytes are shown in Figure 1.

The most striking difference in the behavior of the two lectins, already reported,[2] is that sugars of Mäkelä's group 2 have the ability to make N-specific *Bauhinia* preparations which are nonspecific as a result of a deficiency of inhibitory carbohydrates or as a result of removal of these carbohydrates by dialysis. An example of this is shown in Table 1. In being thus made specific by the presence of certain carbohydrates *Bauhinia* seems to be exceptional.[1]

Table 1 also illustrates a point to which Boyd, Everhart and McMaster[2] did not draw much attention, namely, that sugars of group 2 not only inhibit the "nonspecific" agglutinating activity of *Bauhinia,* but to some extent its anti-N activity too. This is shown better by the example chosen for Table 1, where the concentration of lactose which inhibits the anti-N activity is only eight times the concentration which inhibits the "nonspecific" activity, than by the results of tests with other sugars, not shown. For melibiose, for example, the ratio of the two concentrations is 32.

In no case did the addition of a substance to the *Ricinus* extract cause it similarly to act preferentially on the erythrocytes of N and MN individuals, or on the cells of any other particular individuals. In this sense *Ricinus* is truly "nonspecific."

Discussion

The first impression gained from Figure 1 is perhaps that the various compounds studied have roughly the same inhibiting power for the two lectins, but a closer examination reveals that the compounds referred to in the figure fall into three different groups: (a) A group of substances that inhibit both lectins well, but which in general inhibit *Bauhinia* better than *Ricinus* (compounds 1-7); (b) a group that inhibits *Bauhinia* but does not inhibit *Ricinus* at all (compounds 8-14); and (c) a group that inhibits both *Bauhinia* and *Ricinus* well, although not quite as well as compounds of group (a), and on the whole tends to inhibit *Ricinus* better. It is surely no accident that the compounds in group (a) all belong to, or contain a monosaccharide belonging to, Mäkelä's group 2 (D-mannoheptose, in spite of its name, is seen when written in the pyranose form to have the configuration at carbons 3 and 4 that puts it in Mäkelä's group 2). The compounds in group (b) similarly belong to Mäkelä's group 3 (D-erythrose resembles the group 3 sugars D-glucose and D-mannose when viewed from the nonreducing end). The compounds in group (c) belong to Mäkelä's group 4 (i-inositol, which since it is not a sugar is hard to classify, has at one point the same alternation of upward pointing and downward pointing hydroxyl groups as do monosaccharides of group 4).

Study of Figure 1 makes is very tempting to suggest that a sugar of Mäkelä's group 2

plays an important role in the structure of the receptor with which *Bauhinia* and *Ricinus* lectins combine, and may in fact be a terminal group of this receptor. The inhibitory activity of the "unnatural" sugars of group 4 is more surprising, but suggests that a sugar of this group may play a role in the structure of the receptor, though perhaps not as a terminal group. This sugar might perhaps be rhamnose, a sugar already well known in nature, but it should be remembered that in the case of the Rh blood group antigens, where we were also led to suggest that a group 4 sugar plays a role, we did not think it likely that the sugar was rhamnose.[5]

In contrast to the results obtained with the compounds of groups (a) and (c), the compounds of the middle group (numbers 8-14, or class (b), in Fig. 1) reveal clear-cut differences between the two lectins. *Bauhinia* is inhibited by these substances (which belong to Mäkelä's group 3), but *Ricinus* is not. This would suggest that whereas the "*Bauhinia*" receptor in the red cell contains a sugar of group 3, the "*Ricinus*" receptor does not, or if it does the group 3 sugar is so far from the end of the active portion of the receptor that it has little effect on the specificity. If this is so, then the two receptors, so similar in many ways, cannot be identical.

The fact that D-galactose is nearly as good an inhibitor, for either *Bauhinia* or *Ricinus*, as di- and trisaccharides in which galactose is the terminal monosaccharide might suggest that, accepting for the moment the notion that D-galactose is the terminal unit in the cell receptor involved, the linkage of the galactose in the receptor is not the same as in compounds 2, 3, or 4, or the next-to-terminal sugar is different. Considering that in all the three compounds 2, 3, and 4 the next-to-terminal sugar is glucose, a group 3 sugar, and considering that *Bauhinia* is inhibited by a number of compounds that terminate in glucose (numbers 9-12), this may suggest that it is the linkage that is different.

It will be noted that not only is the nonspecific activity of *Bauhinia* inhibited by galactose and sugars terminating in galactose, but its anti-N activity is also inhibited somewhat by these substances. It is hard to say whether this fact indicates that D-galactose plays some role in the structure of the N receptor. If it does, this would seem to be a further clue to the chemical nature of this blood group antigen.

The results of an inhibition study of the "peanut receptor" (Gy) have been presented elsewhere,[4] and one of us[1] has pointed out how these results and our earlier study of *Bauhinia*[2] suggest that there may be present in the human red cell at least three different receptors terminating in D-galactose (the blood group B receptor, the "*Bauhinia*" receptor, and Gy). It was suggested that in the Gy receptor the next-to-terminal sugar is glucose, linked to the terminal galactose possibly by the 1-1 linkage. It will be remembered that D-glucose and two sugars terminating in D-glucose, melezitose and sucrose, inhibited our peanut extracts but do not inhibit *Bauhinia* or *Ricinus*. L-fucose does the same, but probably for a different reason, for it belongs to Mäkelä's group 1. It will be remembered that L-fucose has regularly been found to be a constituent of the ABH blood group substances.

Perhaps the most interesting idea that occurs to us as a result of this work is the concept that human erythrocytes of persons of all blood groups have receptors containing a sugar of group 2, possibly as a terminal unit, and that this receptor contains also a sugar of group 4. The ready agglutinability of human erythrocytes by *Bauhinia* and *Ricinus*, especially the latter, suggests that the surface of the erythrocyte is well provided with these "nonspecific" receptors, which moreover would seem on the basis of the studies reported here to be of at

least two different kinds. We believe this concept is compatible with the picture of the erythrocyte surface as studded with a variety of receptors, mostly oligosaccharide in nature, and with the notion that only certain of these, and possibly then only the terminal few monosaccharides, are different in individuals possessing different assortments of the alternative genes, A, B, O; M, N; C, c; etc. If this picture at all corresponds to reality, it becomes even more understandable why it has always proved so difficult to isolate specific blood group substances from the erythrocyte.

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The Phenotype Lu(a–b–) Together with Unconventional Kidd Groups in One Family

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Minus-minus blood group phenotypes are characterized by the absence of any detectable phenotypic representation of the alleles expected at the determining gene loci. Double minus phenotypes have been found in most blood group systems but all individuals thus far tested for the Lutheran factors have been Lu(a+b+), Lu(a–b+) or Lu(a+b–). Six individuals with the hitherto undisclosed blood group Lu(a–b–) were found in three generations of a Caucasian family.

Unlike the other minus-minus phenotypes, to which recessive genetical backgrounds have been assigned, Lu(a–b–) appears to be a dominant character.

The Kidd groups of the family are also anomalous: The proposita and her two brothers are Lu(a–b–), Jk(a+b–) yet their father is Lu(a–b–), Jk(a–b+) and their mother is Lu(a–b+), Jk(a+b–). Possible genetical backgrounds are discussed.

THE PHENOTYPE Lu (a–b–) was recognized for the first time in the American family of British origin about to be described. Such "minus-minus" phenotypes, as they have been called, are known in the ABO, MNSs, Rh, Kell, Duffy and Kidd systems; but Lu (a–b–) is of particular interest because it is evidently a dominant character and so differs from these other negative phenotypes for which only recessive genetical backgrounds have so far been invoked.

The family is further gifted with unusual Kidd groups which may be original or may reflect the heterozygous state corresponding to the supposed homozygous condition Jk (a–b–) found so far only in one Hawaiian (Pinkerton, Mermod, Liles, Jack and Noades, 1959) and in five out of 88 Indians from Northern Mato Grosso, Brazil (Silver, Haber and Kellner, 1960).

The ascertainment was simple, though unusual: one of us (M.N.C.) was found to be Lu (a–b–) and the subsequent family investigation brought to light the Kidd originality. There is no known consanguineous mating in recent generations of the family.

Results

The blood groups of the Cr. family are shown in Table 1. The Lutheran and Kidd phenotypes, in which all the interest lies, are set out in the pedigree (Fig. 1).

Lutheran tests

All samples were tested with at least two anti-Lu^a and at least three anti-Lu^b sera: those from II-1, II-2, III-1, III-2 and III-4 were tested with eight different anti-Lu^a and six different anti-Lu^b sera, by saline or anti-globulin methods. The results were confirmed by absorption and elution tests with three anti-Lu^a and two anti-Lu^b sera. Second samples from these five people, taken six months later, gave the same results, as did many further samples from the proposita.

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No evidence of any Lu^a or Lu^b inhibiting principle could be found in Lu (a–b–) plasma: Lu (a+b+) cells were suspended in an excess of the plasma of two Lu (a–b–) members, overnight, at 37 C., but the antigens remained unaffected. Conversely, no evidence for any Lu^a or Lu^b endowing property could be found in the plasma of normal Lu (a+b+) or Lu (a–b+) persons: Lu (a–b–) cells were incubated in such plasma but they emerged still Lu (a–b–).

Kidd tests

All samples were tested with at least two anti-Jk^a and anti-Jk^b sera: those from II-1, II-2, III-1, III-2 and III-4 were tested with ten different anti-Jk^a and six different anti-Jk^b sera, by saline, anti-globulin, trypsin anti-globulin, Löw's papain or ficin methods. All antisera gave very clear reactions: the results are shown in Table 1 and Figure 1.

Absorption and elution tests were done with one anti-Jk^a serum and confirmed the results of the direct tests. The proposita, III-1, and her sibs III-2 and III-4 are Jk (a+b–) but they are unlikely to be Jk^aJk^a, the genotype which normally corresponds to this phenotype, because their father, II-1, is Jk (a–b+): we assume that they have only one Jk^a gene and this is confirmed by their reactions with the majority of the anti-Jk^a sera which were distinctly weaker than those of their mother, II-2, who is presumably a normal Jk^aJk^a. (For example, against titrations of one anti-Jk^a the three sibs scored 24, 21 and 21 while their mother scored 35). This clear-cut dosage effect was shown also by later samples from the proposita and her mother.

Most of the available anti-Jk^b sera were not as good as the anti-Jk^a in making a dosage distinction. However, when duplicate samples from II-1 and from comparable controls were sent to Milwaukee and to London, quantitative tests strongly suggested that II-1 does not possess two normal

TABLE 1. *The Groups of the Cr. Family*

	ABO	MNSs	P_1	Rh	Lu^aLu^b	K k	Le^aLe^b	Fy^aFy^b	Jk^aJk^b
II-1	A_2	*Ns/Ns*	–	*rr*	– –	– +	– +	– +	– +
-2	A_1	*Ns/Ns*	+	*rr*	– +	– +	– –	– +	+ –
-4	A_2	MN.Ss	–	*rr*	– +	– +	– +	+ +	+ +
-6	O	MN.Ss	+	R^or	– +	– +	– +	– +	– +
III-1	O	*Ns/Ns*	+	*rr*	– –	– +	– +	– +	+ –
-2	A_2	*Ns/Ns*	–	*rr*	– –	– +	– +	– +	+ –
-3	O	*Ms/Ns*	+	R^2r	– +	+ +	+ –	+ –	+ –
-4	A_1	*Ns/Ns*	–	*rr*	– –	– +	– –	– +	+ –
-5	B	*MS/Ns*	+	R^or	– +	– +	– +	– +	+ +
-6	O	*MS/Ms*	+	R^1r or R^1R^o	– +	– +	– +	– +	+ –
-7	O	*MS/Ms*	–	R^1r or R^1R^o	– +	–	– +	– +	+ –
-9	O	MN.S	+	R^2r or R^2R^o	– +	– +	– +	+	+
-10	O	*Ms/Ns*	–	R^2r or R^2R^o	– –	– +	– +	– +	– +
IV-1	A_1B	*MS/Ns*	–	R^or	– –	–	– –	– +	+ –
-2	O	*MS/Ns*	+	*rr*	– +	– +	– +	– +	+ +
-3	O	*MS/Ns*	+	R^or	– +	– +	– +	– +	– +

Antisera used: anti-A, -A_1, -B, -A+B, -H; anti-M, -N, -S, -s; anti-P_1; anti-D, -C, -c, -C^w, -E, -e; anti-Lu^a, -Lu^b; anti-K, -k; anti-Le^a, -Le^b; anti-Fy^a, -Fy^b; anti-Jk^a, -Jk^b.
The cells of the proposita, III-1, were positive with anti-H (from an Oh person), -Ss (-U), -P (from a P^k person), -P+P_1+P^k (from a p person), -Kp^b, -Jk^aJk^b (from a Jk (a–b–) person), -Yt^a, -Vel, -I, -Ge and with two unpublished "public" antibodies. They were negative with anti-Mi^a, -Vw, -Vr, -Mε, -V, -VS, -Kp^a, -Di^a, -Js^a, -Levay, -Wr^a, -Sw^a, -Be^a, -By, -Rm, -Good and with several unpublished "private" antibodies.
The saliva of three members was tested: II-1 and II-2 secrete A and H, III-1 secretes H; in all three salivas Le^a and Le^b are present.

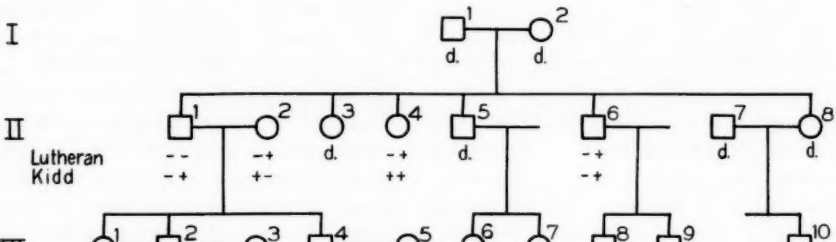

FIG. 1. The Lutheran and Kidd phenotypes of the family Cr. The minus or plus signs of the upper rows represent the reactions with anti-Lu^a and anti-Lu^b: those of the lower rows with anti-Jk^a and anti-Jk^b. Arrow = proposita.

Jk^b genes. Milwaukee scores: II-1, Jk (a–b+), 31; control, Jk (a–b+), 45; control Jk (a+b+), 21. London scores, with a different anti-Jk^b serum: 32, 42 and 20 respectively.)

Other tests

The cells of III-1 were tested with all available "private" antibodies but negative results showed that none of the sera had antibodies for any "new" Lutheran or Kidd antigens the cells might contain. The cells of III-1 were also tested with all available "public" antibodies but positive results showed that none of the sera contained the theoretically possible anti-Lu^{a}Lu^b. A list of these antisera is given in the footnote to Table 1.

No unusual antibodies could be found, by saline or anti-globulin tests, in the sera of II-1, II-2, III-1, III-2 or III-4: none of these people had been transfused.

The proposita is a secretor of H. Her saliva also contains Le^a and Le^b; it does not inhibit anti-Lu^a or anti-Lu^b.

Discussion

The occurrence of two rare groups in one family suggests that there is some association between them, and this will be discussed after separate comments on the two originalities.

The Lutheran groups

Explanations proposed for all other minus-minus phenotypes have had in common the supposition that the conditions reflect a homozygous genetical background. For example the Oh (Bombay) phenotype is believed to correspond to the homozygous absence of a gene *X* which is responsible for the production of H substance from a basic mucopolysaccharide. Other examples are the phenotypes S–s–, Fy (a–b–) and Jk (a–b–) which are thought to be due to the homozygous presence of third alleles, or to the homozygous absence of some necessary modifying gene.

But no such homozygous background can lightly be proposed for the Lu (a–b–) phenotype of the Cr. family. In the statement and treatment of the following problem we are grateful for help received from Dr. F. H. Allen, Jr. Suppose Lu (a–b–) be a recessive character, then the proposita, III-1, being what she is, would require II-1 and II-2 to be carriers and this would further require one of the parents in genera-

tion I, say I-1, to be a carrier. That II-1 is homozygous would require I-2, who was no relation of I-1, to be a carrier also: this would be the first surprising coincidence. But there are two more coincidences: II-7 and III-5, neither of whom are related to the family, would both have to be carriers.

Calculation of how improbable such observations would be if Lu (a–b–) were really a recessive condition would be easy if the frequency of Lu (a–b–) in Caucasians were known. All that we do know is that no example has been found in appropriate tests on nearly 5,000 unrelated Caucasoid people:

	Caucasoids	Negroids
Cutbush and Chanarin (1)	532	
Metaxas, Metaxas-Bühler, Dunsford and Hollander (3)	124	
Kissmeyer-Nielsen (2)	644	
Greenwalt and Sasaki (unpublished)	3,382	286

The finding of the Cr. proposita was, as mentioned above, fortuitous and not a result of organized testing of random samples.

Dr. Allen based his calculations on a range of gene frequencies that would be compatible with finding no homozygote in testing 5,000 people. Thence was calculated the probability of finding three heterozygous carriers among five people married into the family (the other two being the wives of II-5 and II-6). The probabilities range from 0·0008 to 0·00008. Thus, according to these calculations, there is at the most, 1 chance in 1,250 that Lu (a–b–) is indeed a recessive condition.

So, by far the most likely conclusion is that the gene responsible exercises its effect in single dose and that the Lu (a–b–) members of the family have at least one ordinary Lutheran gene which, on grounds of frequency, we must suppose to be Lu^b. Several possibilities suggest themselves. The gene responsible for the Lu (a–b–) condition might be an allele of Lutheran; if so the allele would have to behave in an unconventional way and prevent its fellow allele, presumably Lu^b, expressing itself as Lu^b antigen. If not an allele of Lutheran the gene might be closely or loosely linked to the Lutheran locus, or completely independent of it. Wherever it is sited the gene may be thought of as a suppressor gene blocking the synthesis of Lu^b substance or converting some Lu^b precursor substance to its own use (if the latter, a new antigen might be anticipated).

The Kidd groups

Their offspring show that either II-1 or II-2 must have an unconventional Kidd genotype, and the serological evidence is strongly in favor of it being II-1.

A gene, at present called *Jk*, has been described[4, 5] which does not produce an antigen capable of reacting with anti-Jk^a or anti-Jk^b. Such a gene would, if possessed by II-1, allow a straightforward fitting of genotypes to the Kidd phenotypes of the pedigree: II-1 and IV-3 would be Jk^bJk, III-1, -2, -4 would be Jk^aJk and IV-1 either Jk^aJk^a *or* Jk^aJk. The frequency of the gene *Jk* in Caucasians is unknown; it may be as high as one or two per cent. Only tests with anti-Jk^a and anti-Jk^b on random samples can disclose homozygotes and not enough have been done to exclude a frequency of this order. Heterozygotes, on the other hand, can be detected in family studies; and a Finnish family has recently been tested (in the Blood Group Research Unit) in which there are Kidd irregularities which can be explained most reasonably by assuming the presence of *Jk*.

Had only the Kidd system been upset in the family we would hardly have felt it necessary to look beyond *Jk* for an explanation, but as it is, another possibility has to be considered. If a modifying gene exist, not an allele of Jk^a and Jk^b, able to prevent the expression of Jk^b as an antigen in the heterozygote, Jk^aJk^b, but not in the homozygote, Jk^bJk^b, then genotypes can be fitted to the phenotypes.

Have the two conditions a single cause?

If the Kidd peculiarity be indeed due to the presence in the family of *Jk*, then *Jk* cannot be the Lutheran inhibitor gene (because IV-3 would have *Jk* but has uninhibited Lu^b antigen and, because the homozygote *JkJk*, of Pinkerton, *et al.*, was Lu (a—b+)). Again if *Jk* be indeed responsible for the Kidd peculiarity then there is a faint hint of linkage between Kidd and Lutheran inhibitor gene (four non-crossovers, III-1, -2, -4 and IV-2, to one cross-over, IV-3). There is evidence that Kidd is at any rate not closely linked either to the Lutheran or secretor locus.

The two conditions can be explained most economically as far as genes are concerned, but with the expense of some imagination, in the following way. The Lutheran observations seem to demand a gene having the dominant effect of inhibiting the production of Lu^b: this gene may or may not be an allele of Lutheran. If this gene is to control the Kidd condition then it must also be allowed the power to inhibit the production of Jk^b in the genotype Jk^aJk^b (III-1, -2, -4 and IV-1) but not in the genotype Jk^bJk^b (II-1 and III-10). If a child of III-2 and III-3 were Lu (a—b+) and Jk (a+b+) it would lend support to the idea that a single gene may be behind both the Lutheran and Kidd peculiarities in this family.

Acknowledgments

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A Critique of the Theoretical Hazard of Inter *vs.* Intra-Racial Transfusion*

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From the frequencies of the red cell antigens of Caucasians and American Negroes, the possibilities of antigenic exposure from inter and intra-racial transfusion of blood were calculated. The figures obtained were then modified to take into consideration the relative potency of the individual antigens as well as the behavior of their antibodies. The results lend no support to the alleged advantage of intra-racial over inter-racial transfusion, since the order of magnitude of the antibody stimulation risk from all four kinds of transfusion is the same. From the standpoint of patient safety, emphasis should be placed on the performance of a sensitive compatibility test, rather than on a consideration of the racial origin of either the donor or the recipient.

IN the practice of blood transfusion, it is desirable to minimize the chances for a given individual to form red cell isoantibodies because of the hazard associated with subsequent transfusion or pregnancy. Fortunately, when a sufficiently sensitive serological test is performed, such antibodies can nearly always be detected. Indeed, the low incidence of hemolytic transfusion reactions in areas serviced by adequate laboratory procedures provides excellent testimony for this statement. Furthermore, the general practice of Rh typing both donors and recipients effectively reduces the chances of isoantibody production, since the Rh antigen, D, is by far the most potent of the antigens outside of the ABO system. Except in special cases, further matching of blood types is both impractical and unnecessary, because of the relative weakness of the remaining antigens and limitations in the supply of blood donors.

Due to the great multiplicity of erythrocyte antigens, it is virtually impossible to provide a patient with blood exactly like his own. However, there have been sporadic suggestions that in order to minimize the likelihood of antibody formation, patients should be given blood obtained from donors of the same racial group. Even more specifically, it has been claimed that blood from Negro donors should not be used for transfusing Caucasians, and *vice versa*.

Isoantibodies can only be formed in response to the introduction of antigens not possessed by the patient himself. Therefore, the possibility of providing such a stimulus is proportional to the number of differences in the antigens of donor and recipient red cells. From the known frequencies of the various blood group genes in the two populations, one can calculate the number of possible antigenic stimuli resulting from *inter* and *intra*-racial transfusion. These figures must then be modified in order to provide for the variability in antigenic potency as well as the behavior of their respective antibodies.

It is the purpose of this paper to present the results of such calculations in order to substantiate a recent authoritative but undocumented statement[10] regarding the fallacy of segregating blood donors on the basis of racial origin.

Calculations and Results

From previously published data,[2, 3, 7, 9, 11] the frequencies of the blood group genes of two populations – western European and

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TABLE 1. *Blood Group Gene Frequencies in Two Populations and the Proportion of Each Population Possessing (+) or Lacking (—) the Corresponding Antigen*

GENE	Caucasians			American Negroes		
	Frequency	+	−	Frequency	+	−
A	.279	.480	.520	.172	.315	.685
B	.061	.119	.881	.114	.214	.786
M	.532	.781	.219	.498	.748	.252
N	.468	.717	.283	.502	.752	.248
S	.327	.548	.452	.150	.278	.722
s	.673	.893	.107	.798	.959	.041
P_1	.540	.789	.211	.836	.973	.027
$C + C^w$	.433	.679	.321	.179	.326	.674
c	.567	.813	.187	.821	.968	.032
$D + D^u$	.590	.832	.168	.713	.917	.083
E	.155	.286	.714	.107	.203	.797
e	.845	.976	.024	.893	.989	.011
V	.00?	.00?	.99?	.156	.288	.712
Lu^a	.039	.077	.923	.020	.040	.960
Lu^b	.961	.999	.001	.980	.999	.001
K	.046	.090	.910	.010	.020	.980
k	.954	.998	.002	.990	.999	.001
Le	.755	.940	.060	.528	.777	.223
Fy^a	.414	.657	.343	.053	.104	.896
Fy^b	.586	.829	.171	.122	.229	.771
Jk^a	.514	.764	.236	.732	.928	.072
Jk^b	.486	.736	.264	.268	.464	.536
Js^a	.000	.000	1.000	.103	.196	.804

American Negro—were tabulated, as shown in Table 1. Using these figures, the proportion of each population having either a single or double dose of the given allele (indicated by a plus sign) and the proportion lacking it (indicated by a minus sign) were calculated. To determine the possibility of exposure to a given antigen, it was only necessary to multiply these two figures, representing the donor and recipient population, respectively. A sample calculation (for the antigen S) is shown in Table 2.

Paradoxically, the most important antibodies concerned with transfusion reactions (*i.e.*, anti-A, anti-B and anti-D) can be deleted from further computations, since ABO and Rh typing is routinely performed on the blood of both donor and recipient.

As a result of the bias introduced by giving Rh negative blood to Rh negative recipients, minor changes occur in the exposure probabilities associated with the

TABLE 2. *Method of Calculating the Probability of Antigenic Exposure from Inter and Intra-Racial Transfusion, Using the Antigen S as an Example*

Incidence of Antigen S:

Caucasian		Negro	
S-positive	.548	S-positive	.278
S-negative	.452	S-negative	.722

Probability of exposure to S (as a potential antigenic stimulus) when blood is transfused:

From	Caucasian to Caucasian:	$.548 \times .452 = .248$
	Caucasian to Negro:	$.548 \times .722 = .396$
	Negro to Caucasian:	$.278 \times .452 = .126$
	Negro to Negro:	$.278 \times .722 = .201$

TABLE 3. *Probability of Exposure to Individual Antigens as the Result of Inter and Intra-Racial Transfusions** (Excluding Antigens A,B, and D)*

Antigen	Cauc. → Cauc.	Cauc. → Negro	Negro → Cauc.	Negro → Negro
M	.171	.197	.164	.188
N	.203	.178	.213	.186
S	.248	.396	.126	.201
s	.096	.037	.103	.039
P_1	.166	.021	.205	.026
$C + C^w$	.218	.457	.105	.220
c	.152	.026	.181	.031
E	.204	.228	.145	.162
e	.023	.011	.024	.011
V	.000	.000	.288	.205
Lu^a	.071	.074	.037	.038
Lu^b	.001	.001	.001	.001
K	.082	.088	.018	.020
k	.002	.001	.002	.001
Le^a or Le^b	.056	.210	.047	.173
Fy^a	.225	.589	.036	.093
Fy^b	.142	.639	.039	.177
Jk^a	.180	.055	.219	.067
Jk^b	.194	.394	.122	.249
Js^a	.000	.000	.196	.158
Total × 100**	243	360	227	225

* Method of calculation outlined in Table 2.
** See text for explanation of this figure.

other Rh antigens, particularly c and E. However, since inclusion of these changes in the data cannot be based on a realistic blood banking operation, they will not be considered further.

The data in Table 3 express the individual probabilities of antigenic exposure when the blood of random donors belonging to one racial group is transfused into random recipients of the same or the alternative race. Multiplied by 100, each figure then indicates the number of times one would expect to encounter the respective antibody in 100 transfused patients, assuming the antigen to be 100 per cent effective in evoking a response. Summation of these products, at the base of each column, gives an index of potential antigenic exposure. With the exception of the Caucasion to Negro transfusions, these figures are remarkably similar.

Such data have very little meaning, however, because they fail to take into consideration the fact that some antigens are much more likely to stimulate antibody formation than others. Furthermore, some relatively common antibodies which are usually referred to as "naturally occurring" are rarely responsible for significant red cell destruction. Most examples of anti-P, anti-M, anti-N, anti-Lu^a and anti-Lewis antibodies can be included in this category. Finally, poorly understood differences in the immunologic response of individual patients make it impossible to predict accurately how many exposures to a given antigen may be necessary to stimulate antibody production, or indeed, whether any number of stimuli will be effective.

In order to obtain information concerning the relative potency of the various antigens, data were combined from the reports of four different laboratories[4, 5, 6, 13] on the actual number of antibodies encoun-

TABLE 4. *List of Antibodies (Excluding Anti-D, C + D and D + E) Reported from Four Laboratories*[4, 5, 6, 13]

Anti-K	163
Anti-Le^a and Le^b	162
Anti-E*	137
Anti-c	124
Anti-Fy^a	20
Anti-P	17
Anti-M	12
Anti-C (or Ce)	9
Anti-e	5
Anti-Jk^a	5
Anti-S	4
Anti-Jk^b	2
Anti-s	1
Anti-k	1
Others**	9
Total	671

* The figure for anti-E was partially estimated, since one of the reports did not indicate how many antibodies occurred in combination with anti-D.

** Includes anti-V, anti-U, anti-Js^a, and anti-Vw, etc.

tered in large series of patients and donors. These are tabulated in Table 4. Excluding anti-D, anti-C + D and anti-D + E, 671 antibodies were detected. By far the most common were anti-K, anti-Lewis (*i.e.*, anti-Le^a and/or anti-Le^b), anti-E and anti-c. For reasons given above, anti-Lewis, as well as the other low-titered "naturally occurring" antibodies were not included in the remaining calculations.

Anti-K was most frequently encountered. The exact potency of the K antigen is not precisely known. However, various data[8] indicate that it might be expected to stimulate anti-K formation in about 5 per cent of transfusions involving a K-positive donor and K-negative recipient. The potency of the other antigens can be compared with that of K, by relating the chance of exposure to the frequency of the antibody.

A sample of such a calculation is shown in Table 5. Reference to Tables 3 and 4 indicate that although the probability of exposure to E as an antigenic stimulus is nearly 2.5 times as great as exposure to K, anti-E antibodies are encountered only 84 per cent as often as anti-K. Thus E can be considered to be about one-third as potent as K. This figure is multiplied by .05 to equate it with the estimated effectiveness of K, as described in the previous paragraph.

Similar calculations were performed with nine other red cell antigens, the results being entered in column R of Table 6. The product of R times P, which is the probability of exposure (from Table 3), gives the probability that a given antigen will stimulate antibody formation. For example, in 100,000 random transfusions of blood from one Caucasian to another, one might expect that 410 recipients would form anti-K antibodies.

These individual products of R times P were then subtracted from one, and the remainders were multiplied in succession (to obtain the total probability of non-immunization). This cumulative product, subtracted from one, is given at the bottom of each column in Table 6. It represents the probability that a given transfusion will result in the production of one or more antibodies in the recipient.

TABLE 5. *Method of Calculating Relative Antigenic Potency, Using the Antigen E as an Example*

Antibody	No. reported*	Prob. of exposure**	Estimated potency
Anti-E	137	.204	X
Anti-K	163	.082	.05

$$X = \frac{137 \times .082 \times .05}{163 \times .204} = .0169$$

* See Table 4.

** See Table 3. Since the population of this country is largely Caucasian, the exposure probability for that racial group is used in this calculation.

TABLE 6. *Estimated Potency of 11 Red Cell Antigens and the Probability of Stimulating the Formation of Their Antibodies (Individually and Collectively) by Inter and Intra-Racial Transfusion*

Antigen	R**	P* × R (Probability of antibody stimulation)			
		Cauc. → Cauc.	Cauc. → Negro	Negro → Cauc.	Negro → Negro
K	.0500	.00410	.00440	.00090	.00100
c	.0205	.00312	.00053	.00371	.00064
E	.0169	.00345	.00385	.00245	.00274
k	.0150	.00003	.00002	.00003	.00002
e	.0056	.00013	.00006	.00013	.00006
Fy^a	.0023	.00052	.00135	.00008	.00021
C	.0011	.00024	.00050	.00012	.00024
Jk^a	.0007	.00013	.00004	.00015	.00005
S	.0004	.00010	.00016	.00005	.00008
Jk^b	.0003	.00006	.00012	.00004	.00007
s	.0003	.00003	.00001	.00003	.00001
Cumulative product of 1−P × R		.98812	.98898	.99232	.99188
1 − cumulative product***		.01188	.01102	.00768	.00512

* Values for P (probability of antigenic exposure) were obtained from Table 3.

** R = relative potency of antigen. See Table 5 for method of computation.

*** 1 − cumulative product = the probability of stimulating one or more of these antibodies as the result of a transfusion of the kind indicated.

Discussion

A comparison of the final calculations of *inter vs. intra*-racial transfusion risks indicates that they are all of the same order of magnitude. The slightly greater safety of utilizing Negro over Caucasian donors is probably more apparent than real, since the potency of three antigens, V, e^s and $Js^{a\ 1,\ 3,\ 12}$—found almost exclusively in Negroes—cannot be evaluated at present.

It is doubtful that a single blood transfusion is associated with even a one per cent risk of red cell isoimmunization. The estimates of antigenic potency used in the calculations were based on numerical comparisons of antibodies stimulated by an unknown number of transfusions and pregnancies. Thus, the important factors of primary *vs.* secondary stimulus, as well as the manner of exposure were not considered. Although the hazards contributed by some examples of "naturally occurring" antibodies—particularly anti-Le^a—were necessarily excluded, their effect on the overall risk must be minor.

With these points in mind, it is clear that a small, but presently unavoidable, risk of isoimmunization accompanies every blood transfusion. Therefore, proper emphasis must be placed on the performance of a sensitive and reliable compatibility test, regardless of the racial origin of either the donor or the recipient.

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Panel Discussion

The Minor Crossmatch

The field of blood transfusion is not unique in being blessed with an abundance of unanswered and controversial problems. Periodically we plan to use these pages for presenting panel discussions on selected topics by authorities who have accepted the editor's invitation "to stick their necks out." Readers' comments and suggestions will be welcomed.

To do or not to do a minor crossmatch is a disputed matter and will have to be settled some day. We hope the discussion by our panelists will serve to this end.

The Panel

Ivor Dunsford ..... Sheffield
Eloise R. Giblett ..... Seattle
James F. Mohn ..... Buffalo
B. P. L. Moore ..... Toronto
Kurt Stern ..... Chicago

1. *Do you believe that it is essential to perform a minor crossmatch?*

Dr. Dunsford: No, providing that (a) the ABO groups of both donor and patient are determined by examination of sera and cells and preferably independently by two technicians, (b) the method employed will detect all known sub-groups of A and B, *i.e.,* the routine use of O serum and O cells and microscopically reading, and (c) the method includes a sensitive antibody screening test.

Dr. Giblett: I believe it is desirable to perform a minor crossmatch as a check on the ABO group of the donor and recipient, and to detect polyagglutinability of the recipient's red cells.

Dr. Mohn: No. It has been stated that antibodies in donor plasmas, other than those of the ABO system, are an almost unknown cause of hemolytic transfusion reactions.[1] In a survey of the medical literature between 1948 and 1955 only two reports were found of hemolytic reactions resulting from irregular isoantibodies both following transfusion of anti-T antibodies into recipients whose erythrocytes exhibited polyagglutinability.[2] Many advocates of the minor crossmatch utilize it in lieu of routinely carrying out a serum check of the ABO group of the donor. There is no justification for substituting this test for a complete determination of ABO groups by both cellular and serum properties.

Dr. Moore: As far as transfusions of homologous group are concerned, the compatibility test used and recommended by this Service has never included a test on the donor serum or plasma. To us, the term "minor-match" denotes no more than a check on the donor isoagglutinin *titer* before certain transfusions of heterologous group, because our concept of a compatibility test holds that: (1) Detection of errors in the donor ABO group is within the compass of a good compatibility routine. (Errors in the recipient's ABO group will not always be detected in this way; but greater care in grouping and the use of Cell and Serum Tube Tests will minimize the chance of error.) (2) Detection of auto-antibodies is the function of the auto-agglutinin controls, and when time permits, of a direct Coombs' test on the recipient's cells. (3) The detection of irregular donor antibodies should have been done at an earlier stage.

2. *Have you encountered any instances in which the minor crossmatch was instrumental in averting a transfusion accident?*

Dr. Dunsford: No. This question was posed to several colleagues in different laboratories and although none uses the minor compatibility test, they know of no reaction that could have been prevented by its use.

Dr. Giblett: We have encountered two cases in which the recipient's red cells were strongly agglutinated by the serum of a large percentage of donors. Although it is difficult to be certain that such polyagglutinability will result in significant *in vivo* red cell destruction by anti-T antibodies in the donor plasma, one is reluctant to expose the patient to such a hazard. A severe hemolytic episode, apparently due to anti-T antibodies in transfused blood, has been described in two infants with polyagglutinable cells.[2] Thus the potentiality of erythrocyte damage, even in adults, certainly exists.

Dr. Mohn: No. The present blood bank organization and transfusion service at the university-affiliated general hospital with which I

am associated was established in 1941. From 1942 through 1960 a total of 76,380 pints of whole blood has been transfused in this institution without employing a minor crossmatch in any case. During this period there were no transfusion accidents recorded which might have been prevented if the minor crossmatch had been included as a part of the routine pre-transfusion testing procedure.

Dr. Moore: Our file of reported reactions gives no indication that any of them could be attributed to the presence of an irregular donor antibody.

Authentic cases of reactions due to donor isoagglutinins are hard to find; we know of none apart from several possible cases in infants following exchange transfusion with group O blood.

3. *If you recommend retaining the minor side of the routine crossmatch, outline the manner in which it is done in your laboratory.*

Dr. Dunsford: It is not recommended. If it were, a saline, albumin and antiglobulin technic would be advised. Furthermore, if the minor compatibility test for blood were recommended, then the recommendations would also have to apply to plasma transfusions. One advantage that could be claimed for performing the minor compatibility test by the antiglobulin technic is that it would reveal cases of acquired hemolytic anemia. The number of cases detected in this manner would not justify the routine application of an antiglobulin minor compatibility test and in any case, surely a patient who has an anemia (the cause of which is unknown or in doubt), should have as part of the routine tests, a direct antiglobulin test before transfusion is considered.

Dr. Giblett: Since the donor's serum has already been screened for antibodies, our minor crossmatch is designed primarily to demonstrate agglutination by anti-A, anti-B and anti-T. Therefore, the procedure is carried out on a slide at room temperature with a drop of donor serum and a drop of serum-suspended recipient cells. The mixture is observed microscopically after two minutes and again after twenty minutes (avoiding drying-out with a Petri dish cover).

Dr. Mohn: No comment.

Dr. Moore: Until about six years ago we tested serial dilutions of donor serum against the recipient's cells when transfusions of heterologous group were necessary; the maximum acceptable titers by a sedimentation technic were 1/128 for adults, 1/16 for infants. Since that time, however, heterologous group transfusions have become infrequent, and we have not deemed a "minor-match" (our connotation) necessary for adult recipients. On the rare occasions when such a transfusion is essential, it seems adequate to match O "hemolysin-free" whole blood (or preferably sedimented cells) for A or B recipients, and A of the same subgroup for AB recipients. In the case of infants, the situation is a little different; here sedimented or packed cells are ideal, otherwise O "hemolysin-free" blood with a plasma titer of less than 1/30 against the infant's cells at 20° C. (sedimentation technic) should be chosen.

4. *It has been suggested that, if the sera of all donors are screened for the presence of antibodies, the minor crossmatch can safely be eliminated. Outline your views in regard to the merits or drawbacks of this proposal.*

Dr. Dunsford: All donor bloods should be routinely screened for antibodies. This achieves: (a) avoiding the issuing of blood which might be potentially dangerous providing the screening test is sufficiently sensitive and the red cells used contain all of the major antigens; (b) on future donations the collection of the blood for diagnostic serum (even comparatively low titer antibodies can be used as a diluent for the stronger anti-sera); (c) in addition, a suitable endorsed blood group card can be issued and with it a letter to the donor stressing the importance of the findings should they ever become hospital patients or in the case of women of child-bearing age, the importance of blood tests in pregnancy. This is a service to the donor and to other laboratory staff who may have to prepare blood for the donor as a patient or her baby at some future date.

Dr. Giblett: Screening of donor serum does not eliminate the possibility of ABO typing errors nor does it have any association with the detection of aberrant characteristics of the recipient's red cells. For this reason, we perform both procedures (*i.e.*, minor crossmatch and screening of donor serum).

Dr. Mohn: Routine screening of donor sera against test cells containing the essential blood group antigens by all of the serologic procedures necessary to detect potentially destructive antibodies can involve a considerable amount of additional work and no little expense to a blood bank. Only the larger blood banks may have their own readily accessible sources of standard red cells containing the most impor-

tant blood group antigens and only those staffed with personnel thoroughly trained in blood group immunology are usually sufficiently experienced in all of the technics required for the detection of the various serologic varieties of blood group antibodies which may occur in the sera of donors. The currently available pools of red cells from commercial sources may not always prove to be satisfactory for this purpose. The incidence of irregular blood group antibodies in the sera of a routine donor population would be one of the important indications for the need to perform routine screening. A recent survey of large community or regional donor centers in this country and Great Britain brought forth statistics indicating an over-all frequency of such antibodies ranging from 0.15 per cent to 0.4 per cent.[3] Comments were received from two British centers where only the sera of unselected Rh negative or of female Rh negative donors were examinted for anti-D antibodies, the rates being 0.57 per cent and 2.0 per cent respectively. The latter figure was felt by its observer to have been an inflated one based on the fact that the immunized women detected by the prenatal testing service in his blood bank were invited to become regular donors. For the smaller hospital blood bank, therefore, the additional technical procedures involved in the detection of such antibodies in only a very small segment of the donor population is of questionable value if their performance interferes with the appropriate concentration on the conduct of accurate blood group determinations and reliable comprehensive compatibility tests on the major side.

Dr. Moore: It is generally accepted that a screening test of donor sera for irregular antibodies should be done; the point in question is where it should be done. In our opinion such a test should form an integral part of the initial processing, whether the laboratory be in a regional center or hospital blood bank. Two reasons can be given: firstly, Rh negative blood should be labeled as such only when it does not contain Rh antibodies; secondly, the initial processing of blood should include every practicable test and check so that at a later stage the technician is free to concentrate upon the basic issues of the compatibility test.

Although most of the antibodies found by the screen-test are of no clinical significance, and determination of their specificity and titer is time-consuming, the occasional discovery of high-titer antibody is sufficiently rewarding. Many of the antibodies found in this way are valuable for other laboratory purposes.

5. *Describe briefly the methods which you recommend for routine screening of donor sera.*

Dr. Dunsford: As part of the ABO group, one volume of the donor's serum is tested against one volume of a 2-5 per cent suspension in saline of pooled A cells and pooled B cells (at least six to each pool). The cell serum mixtures are left for two hours at 18-20 C. and then a portion is transferred to a slide and read microscopically. Reactions of any degree, which are unexpected from the red cell antigens, are an indication for further antibody investigations.

In addition, the sera of all donors are tested against papainized pooled O cells—pooled to give as many antigens as possible. It is a modification of the method described by Stapleton and Moore (J. Lab. Clin. Med. 54: 640, 1959). All Rhesus negative bloods are further checked for anti-Kell by testing the serum against O, rr, Kell positive cells in AB serum. The papain technic has proved very sensitive. Recently an anti-K^b (Cellano)* was detected by this method. (Lang, B. and T. Lodge: Vox Sang. To be published.)

Dr. Giblett: A pool of cells obtained from no more than four (preferably two or three) individuals is selected for the content of antigens, and is pre-tested with weak antibodies of known specificity. If the purpose of the screen is to detect as many antibodies as possible, a saline suspension of cells should be incubated with the serum at three temperatures (cold, room temperature and 37 C.) in succession, necessitating only one tube per serum. Otherwise, incubation at 37 C., followed by an antiglobulin test, is adequate for detecting most incomplete antibodies. An alternative method, which is rapid, less expensive, and particularly useful for (most) Rh antibodies, utilizes either papain (modified by Löws technic) or bromelin. In this instance, the enzyme solution is added to the serum prior to the addition of the red cells. Following incubation at 37 C. for ten to fifteen minutes, the tubes are centrifuged and examined for red cell agglutination. Some antibodies evade detection when enzymes are employed, but it is doubtful that these can be considered hazardous when present in the donor's plasma.

Dr. Mohn: No comment.

Dr. Moore: Most of our regional centers use trypsinized or ficinized cells, but at least one

* See Dunsford, *et al.*: Vox Sang. 4: 148, 1959.—Ed.

prefers a one-stage method employing activated papain. The cells used should be DCe/dce and DcE/dce—one of which is Le (a+)—mixed in equal quantities; the results are read macroscopically after 60 minutes incubation at 37 C. If the volume of work was less, the indirect antiglobulin method would be used with selected cells in order to detect a greater variety of antibodies. For cases requiring the use of an extracorporeal circulation during surgery, the advice of Allen and his colleagues is recommended.[1] A pool of fresh, not inactivated, serum from 5-6 donors is tested against selected cells by the indirect antiglobulin method in addition to the enzyme test just mentioned. The important point in this simplified procedure is to ensure that "the amount of any individual antibody in the test is what it would be if each serum were tested separately."[1]

6. *Is there any significant hazard in transfusing incompatible antibodies in the donor's plasma?*

Dr. Dunsford: Apart from O plasma into not group O recipients, the only documented evidence to suggest transfused antibodies could be dangerous is the experimental work of Jennings and Hindmarsh (Am. J. Clin. Path. **30:** 302, 1958.)

In addition, we know of a case where a baby suffering from hemolytic disease of the newborn due to anti-D was inadvertently transfused with Rhesus negative blood with anti-D present in the plasma. The degree of jaundice was so increased that further replacement transfusions were necessary.

Dr. Giblett: I believe that the hazard exists, but is small. The recent studies of Mohn and his associates[1] have shown that unusually potent anti-D antibodies can cause a severe hemolytic process in Rh positive recipients. However, Rh negative blood is rarely given to Rh positive patients, except in the exchange transfusion of infants.

It is possible that other high-potency antibodies, particularly anti-K, anti-E and anti-c may be capable of similar massive destruction of recipient red cells which contain the appropriate antigen. One may reasonably assume that this risk is most applicable to infants and small children, whose plasma volume is insufficient to dilute the offending antibody. In the event that two or more pints of whole blood are pooled (*e.g.* for cardiac surgery), incomplete antibodies present in one donor can combine with their corresponding antigens from the other donor (s), rendering those cells more susceptible to destruction *in vivo*. Obviously, the extent of that destruction is dependent upon such factors as antibody titer, number of vulnerable red cells, size of the pool, etc.

Dr. Mohn: The results of a study of experimental transfusion of donor plasmas containing blood group antibodies into incompatible normal human recipients have recently been reported.[3] In this investigation, each recipient received an entire unit of ACD plasma selected from donors with antibody titers greater than the probable *in vivo* dilution of a single unit in a recipient's total blood volume. These transfusions included examples of anti-Rh, anti-K and anti-M antibodies of varying titers and representative of different serologic varieties based on their *in vitro* characterization. Although in all volunteers isosensitization of their erythrocytes was deliberately created, no clinical reactions were encountered and no good evidence obtained for any significant loss of red cell mass. These observations indicated that the variety and potency of the warm, incomplete blood group isoantibodies investigated had no measurable deleterious effect on the circulating erythrocytes of the recipients transfused with the equivalent of a single unit of whole blood. There is no reason to believe that these results are not applicable to transfusion therapy of ill patients, provided no intrinsic erythrocyte defect exists which would be the situation with the majority of patients requiring whole blood transfusions in any hospital.

Dr. Moore: If one excludes the obvious danger from ABO incompatibility, the principal remaining risk concerns the transfusion of anti-D to Rh positive recipients. This subject has been studied by Jennings and Hindmarsh[5] and by Mohn and his colleagues;[2, 7] Giblett[4] has briefly reviewed the risks and outlined those cases to whom Rh negative blood containing anti-Rh should not be given. It would appear from this work that a frank hemolytic reaction is most unlikely, although a transient hemolytic anemia may develop. Other high-titer antibodies may be presumed to have a similar effect.

7. *If the minor crossmatch is not used, the donor's pilot sample may be collected in ACD solution. Is there any advantage to using ACD blood instead of a clotted sample?*

Dr. Dunsford: Experimental work carried out in these laboratories in 1948 showed beyond any doubt that the red cell antigens D, C, E, c and Kell were preserved for much longer

periods in ACD mixture than when clotted pilot tubes are used. This work was prompted by the missing of an incompatibility due to anti-Kel using a clotted pilot tube that was six days old. If clotted pilot tubes are used, incompatibilities can be missed by the most senstitive technic when the red cells are beyond a certain age. This would suggest a time limit on clotted samples is essential.

Furthermore, with the use of a wide spectrum antiglobulin reagent, far more difficulties are encountered with clotted pilot tubes due to cold incomplete anti-H than are found with cells in ACD mixture.

Dr. Giblett: Red cells suspended in ACD solution appear to retain their antigenic properties (especially K, Jk^a and Fy^a) longer than the cells present in a clot. However, if ACD-suspended donor cells are used, they must be thoroughly washed before incubating them with the patient's serum, for two reasons: 1. Plasma adhering to the cells can form fibrin strands or clots upon exposure to serum. Cells captured in the fibrin often appear to be agglutinated. 2. ACD, like all anticoagulants, is anti-complementary. If the recipient's serum contains an incomplete antibody which requires complement for its detection by antiglobulin serum, inactivation of that complement may make the antibody undetectable.

Dr. Mohn: In December 1959 our routine pretransfusion testing technics were altered to include the collection of pilot samples of donor blood in ACD solution exclusively for use in compatibility testing. This has been accomplished through the use of sealed and numbered segments of the plastic donor tube (Fenwal). During 1960, 13,100 compatibility tests were carried out with donor cells preserved in this manner. This has turned out to be a highly satisfactory procedure making available donor erythrocytes in as excellent condition as those in the unit throughout the dating period of the blood itself. No longer do we encounter those occasionally difficult to interpret crossmatching tests with donor bloods very near to the twenty-one day expiration date where considerable hemolysis has occurred in the clotted pilot sample and pseudoagglutination or slimy, stringing together of the donor cells is observed in the test. This procedure has also almost eliminated any clerical error in identification of the donor samples used in the laboratory in the crossmatch since they are integral parts of the plastic donor unit itself until removed and bear the number of the unit. A single clotted specimen is still obtained only for examining for the absence of serologic evidences for syphilis and for performing the routine ABO and Rh grouping determinations on the donors.

Dr. Moore: The pilot tubes used in our Service have always contained ACD. There are two important reasons for this choice: (1) Compatibility tests are of limited value if the agglutinability of the donor cells is impaired. Most blood group antigens are still quite effective after 14-21 days in ACD, the main exceptions being c and P.[5] Crawford, Cutbush and Mollison,[3] on the other hand, have shown that after even seven days at 4 C. the reactions of cells from clotted blood may be distinctly weaker than those of fresh cells. (2) If washed cells from citrated blood are used in the compatibility test, it is then possible to use antiglobulin reagents containing potent "anti-complement" components without fear of difficulties from weak false-positive reactions.

It seems to me that the need for ACD in pilot tubes is a fundamental issue and should take precedence over such disputed questions as the need for "minor-match."

8. *Some institutions screen the serum of each patient for the presence of antibodies at the time of admission in order to uncover crossmatching problems in advance. Would you favor widespread adoption of this procedure?*

Dr. Dunsford: Yes. This divides into two parts—the emergency admission and the planned case.

In the emergency admission, a quick screen with a pool of cells containing as many antigens as possible by a sensitive antiglobulin technic should be carried out, or at least by an enzyme or albumin technic. This enables the technician to set up the major compatibility test against more donor bloods than those requested in the hope of finding sufficient for the immediate need.

In the planned case, the antibody detection screen and subsequent identification should be routine to enable coordination of the services for the supply of special typed blood. This planning leads to better feelings between the suppliers and users of the blood and enables better service to the patient and our colleagues in other hospitals (see answer to question 4—para. c). In fact, the findings of immune antibodies may be a contra-indication to further transfusions if these can be avoided.

In our own laboratories a Regional register of known immunized patients or would-be patients is maintained and is proving invalu-

able. Since we also maintain the Regional panel of typed donor bloods, it leads to greater efficiency.

Dr. Giblett: The procedure is not possible in this area, because all of the typing and compatibility tests are performed in the laboratory of one central blood bank. It is our practice to screen the patient's serum at the time of the crossmatch, the procedures being carried out in parallel.

There are obvious advantages in having advance information about the presence of antibodies in a patient's serum. However, it would be unfortunate if a negative "screen" were considered sufficient reason for omitting a careful crossmatch. Screening technics are not infallible, and there is always the possibility that the recipient has an antibody with a corresponding antigen not represented in the screening cells.

Dr. Mohn: I do not favor widespread adoption of this procedure for most of the reasons given for my negative reaction to routine screening of donor sera. Again this would require ready availability of well preserved individual cells or pools of cells containing all of the important erythrocyte antigens. To the smaller hospital blood bank the time and expense involved in carrying this out on a regular basis might be prohibitive. This objection could be overcome in part by limiting such screening to the sera of patients found on admission to have a known history of multiple transfusions during a previous admission in the same or some other institution and likely to be candidates for transfusion again. This would depend upon accurate information of this nature being obtained and transmitted promptly to the blood bank well in advance of any request for a blood transfusion.

Dr. Moore: Some years ago those of our centers that offer an area crossmatching program were recommended to use an indirect antiglobulin screen test of the recipient's serum against a mixture of no more than two fresh cell-samples containing between them the antigens S, D, C, E, c, K, Le^a, Fy^a, and Jk^a. Apart from uncovering a fruitful source of interesting antibodies that are bystanders so far as the donor antigens are concerned, this step was intended to control the possibility that certain donor antigens from 14-21 day blood might not be effective in detecting a weak antibody in the recipient.[6] We also recommended that the serum of cases requiring periodic transfusion should be investigated from time to time for irregular antibodies, so that emergency transfusions need not be delayed or withheld for lack of compatible blood. I doubt whether it would be practicable to perform an antibody screen test on all patients at the time of admission in any but the smallest centers. However it is a sound idea to do this test before the compatibility routine. I know of several Canadian hospitals that feel such a course is an essential safety factor as well as an invaluable source of antibodies for test purposes.

Dr. Stern: The minor crossmatch is an integral, valuable, and readily performed part of pretransfusion tests which serve as safeguards to protect the recipient from some of the most serious hazards of transfusion therapy. Reasons that can be adduced in justification of this statement can be divided into three categories. The *first*, and most important one, concerns *detection of ABO incompatibility*. In this respect the minor crossmatch is a check procedure analogous to the "reverse" or "confirmation" grouping which is generally considered to be essential for determination of the ABO group. Accordingly, the minor crossmatch may reveal technical or clerical errors in ABO groups of donor and prospective recipient that otherwise might escape detection. Jennings and Hindmarsh[9] have pointed out that errors in ABO group detected *only* by the minor crossmatch represent situations in which the blood selected may be considered as "universal donor" or the patient as "universal recipient." However, this does not abolish the significance of such errors, especially since they may not only affect the donor and patient directly involved but, in some instances, may be even of more serious import for utilization of other units of blood or samples of other prospective recipients if the error has resulted from confusion of specimens or similar forms of mistaken identity. Furthermore, in some situations blood incompatible on the major as well as on the minor side may have been selected erroneously, *e.g.*, A blood for a B recipient, or *vice versa*, but the major crossmatch may fail to reveal the incompatibility for one or more of the following reasons: isoagglutinins in the recipient may be of extremely low titer, such as in some patients with leukemias or lymphomas,[5, 11] hypogammaglobulinemia or agammaglobulinemia;[2] or, the reactivity of the donor's red cells may be poor because of age of the pilot tube, inadequate preservation, or presence of a weakly reacting subgroup of A. In such instances the incompatibility in the minor crossmatch will alert the worker to search for the cause of the unexpected agglutination observed and thus use of mismatched blood will be averted. Finally, the

minor crossmatch may reveal unusual complications, such as presence in the donor's serum of anti-A_1 active at room or even body temperature.

The *second* reason is based on the *obligatory nature* of the minor crossmatch in certain specific situations. If such a need arises as emergency, blood bank workers not attuned to the regular performance of the minor crossmatch may be at a serious disadvantage. The most common cause for mandatory performance of the minor crossmatch is associated with use of "universal donors" or transfusion of "universal recipients" outside of their own ABO groups. Improper selection of blood in these situations has frequently been followed by disastrous consequences.[6, 7,8] Sometimes an Rh-negative patient may be transfused with Rh-negative blood containing Rh antibodies. If it cannot be excluded that the patient may have received Rh-positive blood within the preceding weeks, it is essential that the minor crossmatch demonstrate the absence of surviving Rh-positive red cells which could react with the transfused antibody. In general, when it is intended to use Rh-negative blood with Rh antibodies for presumably Rh-negative patients, it is desirable to perform a minor crossmatch in order to rule out presence in the recipient's red cells of D^u, rh′ (C), and rh″ (E), all of which might interact with the donor's antibodies. Obviously, such minor crossmatch tests must utilize technics capable of demonstrating presence of incomplete antibodies (antiglobulin or enzyme tests).

The *third,* and possibly least important, reason for doing minor crossmatch tests is *detection of irregular isoantibodies* in the donor serum as shown by reactivity with the recipient's red cells. As a matter of course, detection of such antibodies critically depends on the technic of compatibility tests used; *i.e.,* only with the help of antiglobulin and/or enzyme methods can one expect to demonstrate incomplete antibodies for Kell, Duffy, Kidd and other blood factors. A special problem is represented by donors with "high-frequency" antibodies. While at first glance it might appear as extremely unlikely that a donor with anti-Tj^a (anti-P + anti-P_1) might become a donor for a random recipient, such an incident *almost* occurred within the last year, thus proving again that blood bank work, just as life in general, may be stranger than fiction. Some time ago we had the opportunity of studying a family with six Tj^a-negative siblings.[3] A few months later a telephone inquiry was received from a small hospital, quite a distance from Chicago, where the father of the Tj^a-negative persons was scheduled for surgery. One of his sons, known to us as Tj^a-negative, was thought to be a suitable donor for his father since both were group O Rh-positive. However, to the surprise of the laboratory staff, hemolysis was observed in the minor crossmatch using the father's red cells and the son's serum. For obvious reasons, it was recommended to use another donor for the patient. With the increasing use of hemotherapy and the broadening of the donor population, incidence of isoimmunization may be expected to rise among blood donors, including presence of "high-frequency" antibodies. Another situation of admittedly exceedingly remote probability is that of a recipient with the extremely rare factor Mg; on the other hand, donors who might have the corresponding antibody are not at all uncommon.[1] In this hypothetical situation only the minor crossmatch can detect the incompatibility.

Several arguments are offered by proponents for elimination of the minor crossmatch. Detection of "minor" incompatibilities is said to be unimportant because transfusion of incompatible antibodies is supposedly of little consequence for the recipient. Apart from the fact that many serious, and even some fatal, transfusion reactions have been recorded as a result of use of "dangerous universal donor" blood,[6-8] this reasoning can be subjected to additional criticism. Experimental studies of Jennings and Hindmarsh[9] and of Culp and Chaplin[4] have disclosed evidence for destruction of red cells as a result of transfusion of some incompatible antibodies. It is ovbiously impossible to predict in each case in advance whether and to what extent such effects will be encountered. Most importantly, one must not lose sight of the fact that the goal of hemotherapy is not merely elimination of serious transfusion reactions, but rather assurance of maximum benefit to the patient. This certainly is *not* accomplished when even a small fraction of the red cells of the patient, whose vital functions are often already impaired, is subjected to accelerated destruction.

Neither can this writer agree to the assertion that the minor crossmatch adds too much complexity to blood bank work. Since red cells of the recipient and serum of the donor must be available for other parts of the pretransfusion tests, the preparation of one additional mixture cannot be considered to impose a serious burden on the technologist. There is also no need for appreciably prolonging the time required for

completion of the test since the same periods of incubation can be used for the major and minor crossmatch tests. The fear has also been expressed that the minor crossmatch test may become a potential source of error because the technologist may prepare the wrong mixture of serum and red cells or misinterpret the final readings. This objection does not appear to have much merit since opportunities for similar mistakes are present in connection with "direct" and "reverse" ABO grouping. A technologist prone to such sources of confusion should probably be best diverted from blood bank work to some other less critical activity.

It is difficult to pinpoint concrete incidents in which the minor crossmatch was instrumental in averting transfusion accidents. Jennings and Hindmarsh[9] included in their report the number and types of irregular isoantibodies detected by means of the minor crossmatch test at the Mount Sinai Blood Center, Chicago. Since then three additional donor antibodies were found: an anti-P_1 with good saline and antiglobulin reactivity, and one instance each of low-titered anti-rh″ (E) and anti-Jk^a. None of these units of blood was used for recipients with the corresponding blood factors.[12]

The technic of the minor crossmatch recommended[12] parallels that of the major crossmatch: (a) a 2 per cent suspension in saline of the recipient's red cells is added to the donor's serum and the mixture centrifuged immediately; (b) if this step has failed to produce agglutination, the mixture is incubated for 15 minutes at 37 C.; (c) in the absence of agglutination after the preceding step, the mixture is converted into an antiglobulin test. For investigative purposes the cysteine-papain crossmatch technic[11] has also been used; results of this work will be presented as a separate report. Whenever blood is used outside of its own ABO group, the minor crossmatch test proposed by Grove-Rasmussen, *et al.*,[8] is to be used.

It is of considerable advantage and most desirable to screen serums of donors for antibodies. The critical question is that of the extent to which such screening should and can be done. We have reported on the use of pooled papain-treated red cells for such purposes,[10] a method which detects almost all Rh and some other types of irregular antibodies. It must, however, be kept in mind that in many small blood banks screening technics are not only difficult to institute but may be also insufficiently reliable because of lack of adequate facilities and experienced personnel. Under such conditions I would strongly object to replacement of the minor crossmatch by screening tests of the donors' serums. Obviously there is also the inherent danger that such reasoning may be applied with seemingly equal justification to screening of the patients' serums as a step permitting elimination of the *major* crossmatch.

Opinions on the hazards associated with the transfusion of incompatible antibodies have been expressed in preceding parts of this discussion.

Collection of donor's pilot sample in ACD solution does not seem to me to be predicated on elimination of the minor crossmatch. Serum of the donor is still needed for antibody screening and for most methods of serologic testing for syphilis. If sufficient evidence is available for better preservation of reactivity of hemoantigens in ACD than in clotted blood, it would appear desirable to collect both types of samples of donor blood.

Screening of the serums of prospective recipients of blood transfusions for presence of irregular isoantibodies is most desirable. A method, such as that of pooled papain-treated red cells,[10] appears to be simple enough to be included in routine pretransfusion tests. In addition to detection of Rh antibodies, this method also readily establishes presence of autoantibodies and thus provides information of considerable importance for elimination of technical sources of error in selection of blood as well as valuable guides for the clinical management of the patient.

Editor's Comment

The panelists' opinions concerning the necessity for performing the minor crossmatch vary from an unqualified "No" to a flat "Yes." The decision to retain or to drop this portion of the crossmatch will have to be made on an individual basis. Its major function seems to be to detect errors in ABO grouping. If the patient's and donor's blood groups are performed properly, then the minor crossmatch is considered of minor importance by the majority of the panelists. Dr. Giblett retains it as a simple slide test "to demonstrate agglutination by anti-A, anti-B and anti-T, since the donor's serum has already been screened for antibodies." Instances in which donor's antibodies have caused overt reactions appear to be difficult to find. The merits of screening patient's and donor's sera for antibodies are generally accepted but not deemed universally feasible.

The ACD donor pilot sample is favored over the usual clotted specimen.

You may be right or wrong in doing or not doing the minor crossmatch. The answer depends on what else you are doing and how well you do it.

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Hemolytic Disease of the Newborn Apparently Caused by Anti-Lu^a

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Report of the first example of pure anti-Lu^a associated with hemolytic disease of the newborn. Of special interest is the fact that this serum demonstrated a marked prozone reaction in saline, papain and indirect Coombs titrations, and is the first anti-Lu^a serum to react well by the indirect Coombs technic.

ANTI-LU^a, and the Lutheran Blood Group System which it defines, was first reported by Callender, Race and Paykoc in 1945.[2] Race and Sanger[4] state that none of the 13 examples of the antibody known to them have been responsible for transfusion reactions or for hemolytic disease of the newborn. They further state that anti-Lu^a has not been reported as an incomplete antibody.

It is possible that cases of anti-Lu^a associated with hemolytic disease of the newborn could have been recognized if the practice of examining cord bloods from all deliveries by the direct Coombs test were generally adopted. This routine, however, is only practiced in a few hospitals. Early in 1959, this test was initiated as a routine procedure at St. Joseph's Hospital, Houston, Texas.[3] Although Stratton and Renton[5] describe a suspected case of hemolytic disease of the newborn due to anti-E and anti-Lu^a, the two cases detected by this procedure and herein described are, to the best of our knowledge, the first association of a pure anti-Lu^a with hemolytic disease of the newborn, as well as being the first example of the antibody which reacts well by the indirect Coombs technic.

Case Reports

First Hospital Admission: In April 1959, a two plus reaction was detected on a cord blood obtained following the sixth delivery of Mrs. Sc., a 29-year-old white woman. The infant exhibited no clinical evidence of hemolytic disease. On discharge, four days later, the direct Coombs test on the infant's cells still gave a two plus reaction. The mother, Mrs. Sc., had no history of blood transfusion, nor had there been any reported difficulty in connection with the delivery of any of the previous five children, despite the fact that three of these children were Lu^a positive.

Both Mrs. Sc. and the infant belonged to group B. Mrs. Sc.'s serum gave a strong positive reaction with her husband's group O cells and also with the infant's cells, after these had been eluted free of the coating antibody using the heat elution technic of Landsteiner and Miller as described in the report of the first 2,000 cord blood specimens of the present series.[3] The eluate from the infant's cells also gave positive reactions when tested against the father's cells.

Blood samples from Mrs. Sc., from her husband and from the infant were sent to the Knickerbocker Foundation, Inc., New York, for further investigation. Mrs. Sc.'s antibody was tentatively identified by them as a pure anti-Lu^a. The blood samples were forwarded to the Lister Institute in London where Drs. Race and Sanger confirmed the identification of anti-Lu^a.

TABLE 1. *Anti-Lu^a Titrations—Four Months After Delivery of Sixth Infant*

1:	1	2	4	8	16	32	64	128	256	512	1,024	2,048
Saline Immediate Spin	0	1+	3+	0	0	0	0	0	0	0	0	0
4 C.	0	1+	3+	3+	2+	1+	0	0	0	0	0	0
22 C.	0	1+	3+	3+	2+	1+	0	0	0	0	0	0
37 C.	1+	2+	3+	3+	2+	2+	1+	0	0	0	0	0
Indirect Coombs	2+	3+	3+	4+	4+	4+	3+	3+	2+	2+	1+	0
Papain*	0	±	1+	2+	2+	2+	3+	2+	±	0	0	0

* Dilutions in AB serum + one volume of papain solution + one volume of 5 per cent red cells in saline—read after 1.5 hours at 37 C.

Second Hospital Admission: In October, 1960, Mrs Sc. was admitted to St. Joseph's Hospital for the delivery of her seventh child. A weak positive direct Coombs test was obtained on the cord blood cells. An eluate prepared from these cells reacted strongly with the father's cells and with one other sample of known Lu (a+b+) cells. The eluate did not react with any of the other 15 selected Lu (a−b+) test cells. Serum from the cord blood did not contain any demonstrable free anti-Lu^a. The infant showed no clinical evidence of hemolytic disease. The bilirubin level of the cord blood was 2.1 mgs. per cent. It rose to a peak of 9 mgs. per cent on the fourth day and returned to a level of 1.0 mgs. per cent on the fourteenth day. The hematologic findings were within the normal range. The infant's direct Coombs test was still positive when last tested on the fourteenth day.

Family Lutheran Typings

Through the generous gift of anti-Lu^a and anti-Lu^b sera from Dr. Fred H. Allen,* we were able to type the Sc. family for the Lutheran system. The results of these typings are shown in Figure 1.

Characteristics of the Antibody

Mrs. Sc.'s serum drawn shortly after the 1959 delivery gave weak reactions to Lu (a+b+) cells and titrations were not done at that time. Four months later a sample was obtained and the titer was determined. The results are shown in Table 1.

A marked prozone was observed in the saline, papain and indirect Coombs titrations. In retrospect, the weak reactions of the first specimen of serum may have been due to this prozone reaction. The anti-Lu^a characteristic "small clumps in a field containing a large number of free cells" was not observed except in those high dilutions giving weak reactions. Titers were performed *vs.* Lu (a+b+) cells. Lu (a+b−) cells were not available and hence no dosage effect determinations could be made.

Results of titrations performed prior to the seventh delivery are given in Table 2. These titrations were also obtained *vs.* Lu (a+b+) test cells. The prozone reaction, observed in the earlier titrations, was not demonstrated in these titrations, nor was the antibody reactive except in those procedures employing indirect Coombs technics.

Discussion

This report of a case of hemolytic disease of the newborn due to pure anti-Lu^a detected by the direct Coombs test on cord blood provides further evidence for the desirability of including this test as a part of the routine procedure for every delivery. The finding of a positive direct Coombs test on cord blood cells is sufficient to make the diagnosis of hemolytic disease of the newborn, regardless of the clinical severity of the disease.[1]

In spite of the fact that Mrs. Sc. had an antenatal antibody titer of 1:4,000, her seventh infant's cord blood cells gave only a weakly positive direct Coombs test. This could be due

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TABLE 2. *Anti-Lu^a Titers During Seventh Pregnancy*

Anti-Lu^a (1960)	May	June	July	Aug.	Sept.	Oct.*
Indirect Coombs	1:1,024	1:2,048	1:2,048	1:4,096	1:4,096	1:4,096

* Titer at time of delivery.

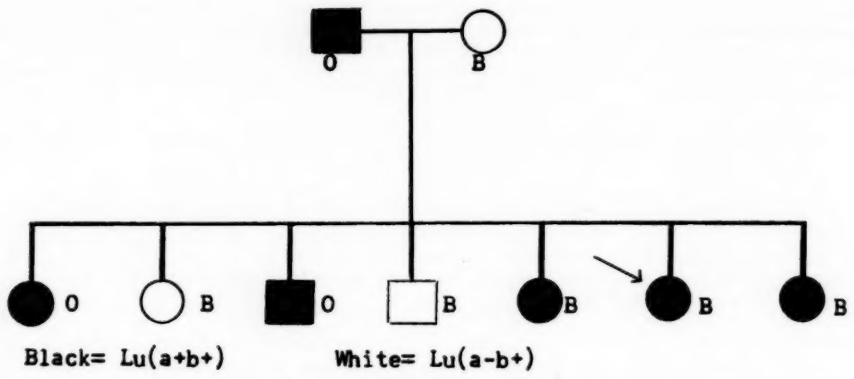

FIG. 1. Sc. Family Lutheran Typings.

to blockage of transplacental antibody migration, decreased potency of the Lu^a antigen (*i.e.* fewer reactive sites) on fetal red cells, or an excess of antibody coating those red cells. In the later instance, the antibody becomes the antigen in the antiglobulin test, so the inhibition of agglutination is actually due to antigen excess. This would at the same time explain the prozones seen on titrating the antibody with adult Lu (a+) cells.

Although it is possible that more cases of hemolytic disease of the newborn caused by anti-Lu^a could have been recognized by the routine performance of the direct Coombs test on cord blood, it seems that these cases would still be very rare. The example cited by this report is the only one recognized in the 7,500 cord blood specimens examined by this procedure at St. Joseph's Hospital since 1959.

Acknowledgment

The authors wish to express their appreciation to Dr. Ivor Dunsford, Sheffield, England, for performing the papain titration on Mrs. Sc.'s serum as shown in Table 1.

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Book Reviews and Abstracts

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Book Reviews

Advances in Blood Grouping. Alexander S. Wiener. New York, Grune & Stratton, 1961, 548 pp. $11.

In 1954, *Rh-Hr Blood Types,* a volume of 84 of his reprints was collected by the author; it is currently out of print. *Advances in Blood Grouping* is an additional collection of Dr. Wiener's papers, most of which were published between 1953 and 1959. The papers have been reprinted from 30 journals and are arranged under 14 general headings.

The title is somewhat of a misnomer in that the author's writings, though extensive, have covered only those fields of blood grouping in which he has an interest. This is not a general summary of recent additions to knowledge of the field.

It seems likely that few workers in blood grouping could have read all these papers in the original. Consequently their being brought together will prove helpful, especially, as the author says, to new workers. Those who wish to understand Dr. Wiener's concepts of blood group inheritance and nomenclature will find this review of particular value.

Although Dr. Wiener is apparently preoccupied much of his time with Rh-Hr and C-D-E contrasts, these reprints will illustrate the wide range of his interests. There are papers on gamma globulin, blood groups and disease, erythroblastosis fetalis, newly discovered Rh factors and autoantibodies among others. On these latter subjects, the imaginative turn of his mind and the careful technics employed in his serologic work are well illustrated. Without doubt Dr. Wiener is one of the giants in his field, and reading this latest volume will prove to be a rewarding experience.—*John B. Ross.*

Bleeding Syndromes. A Clinical Manual. Oscar D. Ratnoff. Springfield, Ill. Charles C Thomas, 1960, 287 pp., 7 tables, $8.50.

In recent years a small number of monographs on the hemorrhagic diatheses have been published. At least three come to mind and each has proved useful as a reference source. Hemorrhagic diseases are sufficiently puzzling that many points remain obscure to all but a few workers and it is frequently necessary to refresh one's memory. While these three references were written for physicians, it is likely that they have found their greatest audience among workers in the hematologic fields.

Bleeding Syndromes is different from other monographs in that it was written for the clinician who wishes to understand the subject without being exposed to a mass of information. The author's bibliography is extensive enough for anyone (800 references) but only a few selected ones are placed after each chapter. The first chapter of ten pages is devoted to the physiology of hemostasis. The second chapter of about 15 pages describes briefly the most important diagnostic features and the most common laboratory tests. The remaining 200 pages are devoted to descriptions of the known hemorrhagic syndromes. The number of syndromes is growing each year, and, as far as the reviewer can tell, Dr. Ratnoff has omitted none of them.

For the most part the author has chosen to discuss each subject in such a way that the attending physician will have a clear understanding of the nature of his patient's disease, what tests may prove useful, what therapeutic agents are available and what each may accomplish. The author has, by a careful choice of material, omitted such things as historical data, discussions of nomenclature and the use of uncommon tests. Easy readability has been accomplished in large part by the author's facile use of the English language. He manages to put a great deal of information into a relatively small number of words.

As hematologic texts go, this is one of the shortest and most readable. It encompasses enough material that it may be used as a textbook by students and house officers. For practicing physicians, *i.e.*, internists, pediatricians, surgeons, general practitioners, etc., it will prove of inestimable value.—*John B. Ross.*

Observations on ABO Incompatibility Between Mother and Infant. G. C. Robinson, H. G. Dunn and L. C. Wong. Suppl. 120, Acta Paediatrica, February 1960, 86 pp.

THIS MONOGRAPH deals largely with the personal observations of the authors based upon serological studies of 109 male and 63 female random group O blood donors; 154 group O prenatal women and their infants; and 37 additional infants referred by others for study. Anti-A hemolysins were found in over 40 per cent of the random donors and in approximately 75 per cent of the prenatal samples regardless of whether the pregnancy turned out to be homospecific or heterospecific. The authors feel that the presence of hemolytic forms of anti-A or anti-B antibodies is not a useful diagnostic test. Eight cases of hemolytic disease were detected in the offspring of the 154 random group O mothers mated with fathers of unknown ABO groups—an incidence of 5.1 per cent or 2.2 per cent of all pregnancies. In the 37 cases of overt hemolytic disease the direct antiglobulin test was positive in 13 of 35 tested with "commercial serum" but 24 of 27 tested were found positive with "native absorbed antiglobulin serum." The red cells were only washed twice in saline. Twelve of these 37 selected cases were treated with replacement transfusions. Diagnostic criteria are given.

While this publication cannot be considered a complete or even up-to-date presentation of the subject, it is recommended to those who have not kept up with the literature. It supplies a sound basic background for an understanding of this form of hemolytic disease of the newborn.—*T. J. Greenwalt.*

Abstracts

Storage and Transfusion

The Use of Glycerolized Frozen Blood in Vascular Surgery and Extracorporeal Circulation. T. G. O'Brien, L. L. Haynes, A. C. Hering, J. L. Tullis and E. Watkins, Jr., U. S. Naval Hosp., Chelsea, Massachusetts. Surgery. 49: 109, 1961.

THIS PAPER illustrates technics in which red blood cells are glycerolized in the Cohn fractionator before freezing and subsequently deglycerolized. The thawed blood was compared with fresh blood by a variety of analytical procedures, and no significant differences were detected.

Detailed case reports are given of patients who received large volumes of thawed red cells suspended in albumin solution or in their own heparinized plasma, the latter being used for open heart surgery in some cases. The authors were impressed by the lack of abnormal bleeding and the general maintenance of platelet counts above 100,000 per cu. mm. Plasma hemoglobin levels of the patients subjected to open

heart surgery rose as high as 300 mgm. per 100 ml. and some postoperative renal impairment occurred. The authors claim similar findings with fresh, heparinized blood, though plasma hemoglobin levels in that range seem greater than most current groups experience.

Use of frozen blood for open heart surgery has the advantage that compatible donations can be selected at leisure and frozen at any point prior to operation. All have to be deglycerolized at once, however; and the authors found it advisable to thaw half of the units the day before surgery.—*H. A. P.*

Double-Helical Reservoir Heart-Lung Machine. N. Zuhdi, B. McCollough, J. Carey and A. Greer. Mercy Hospital, Oklahoma City, Oklahoma. Arch. Surg. 82: 320, 1961.

THE authors describe a technic whereby a simple bubble-type oxygenator is primed only with 5 per cent glucose in water before surgery. No heparinized donor blood is used. Blood lost during surgery is replaced by ACD bank blood stored up to five days. Low flows through the pump-oxygenator are made possible by the use of hypothermia.—*H. A. P.*

Influence of Glucose and Nucleoside Metabolism on Viability of Erythrocytes During Storage. G. R. Bartlett, Scripps Clinic, La Jolla, California. Arch. Int. Med. 106: 889, 1960.

AFTER a brief summary of various approaches used to investigate extension of the storage period of blood for transfusion, the author presents an excellent analysis of studies which have endeavored to normalize the metabolism of stored red cells by the addition of nucleosides. The ability of nucleosides to restore the level of high energy phosphate compounds and increase the per cent of viable red cells is unquestionable. Variation in reported results, lack of knowledge of the precise mechanism of the storage lesion and optimum conditions for nucleoside supplementation require further investigation before the technic can be recommended for routine blood bank use.—*H. A. P.*

Serological Difference Between Old and Young Cells. F. Stratton, P. H. Renton and V. I. Rawlingson, Regional Transfusion Service, Manchester 1, England. Lancet. 1: 1388, 1960.

SERUM from a 71-year-old woman with Hodgkin's disease reacted with cells of all types to an equal degree. The reaction was stronger the longer the test cells had been stored. Red cells reacted more strongly if they had been glycerolized and frozen, or if they had been heated to 56 C. Aging of red cells *in vivo* also appeared to increase their sensitivity to agglutination by this serum. The patient's own red cells reacted similarly but more weakly.—*H. A. P.*

Air Pressure to Speed Up Blood Transfusions. (Letter to the Journal). W. P. Murphy, 1101 Beacon St., Brookline 46, Massachusetts. J.A.M.A. 175: 184, 1961.

THE author has given over 3,000 transfusions using air pressure in the bottle, without adverse reaction or fatality. He believes the key to the whole problem is not the technic used, but the constant supervision of a qualified transfusionist. He points out that transfusion by gravity without adequate supervision increases the risk of fatality if incompatible blood is given. He also quotes one case in which severe extravasation of blood occurred during an unsupervised gravity transfusion.—*H. A. P.*

Correspondence. Air Pressure in Blood Transfusions. W. S. Howland. Memorial Center for Cancer and Allied Diseases, New York, New York. J.A.M.A. 174: 160, 1960.

THIS letter protests a recent editorial which stated that the transfusion of blood under air pressure was unnecessary, dangerous, and should be prohibited. It states that one or more such transfusions a day have been given during the past ten years at Memorial Hospital for Cancer and Allied Diseases. Countless lives have been saved by the procedure and only one death from air embolism occurred. A safety vent trap bottle of saline solution is interposed between the blood bottle and the patient. The author of this letter also feels that the administration of calcium is unnecessary during massive replacement with citrated blood.—*H. A. P.*

Blood Groups and Antibodies

Modern Blood Group Mythology. A. S. Wiener, Office of Chief Medical Examiner, New York City, New York. J. Forensic Med. 7: 166-176, 1960.

DR. WIENER'S paper discusses two aspects of blood groups under the headings, "blood groups and disease" and "Fisher's synthesis and little d." On the first subject he says that studies on "—the association of particular A-B-O blood groups and particular diseases—have no rationale, and lead only to contradictions and fallacies." Several reasons but no statistical data are given for rejecting these associations.

On the second matter, Fisher's linkage theory of the inheritance of several common Rh factors and his renaming them C, D, E, c, d and e are mentioned and criticized. Particular criticism is directed at the authors of three books for citing the premature announcement of the discovery of anti-d in support of Fisher's synthesis.

(The reviewer has read a substantial number of Dr. Wiener's papers in recent years. Many of them are worthwhile contributions to blood group serology in particular and medical practice in general. Unfortunately there are others that shed more heat than light, and this paper belongs in the latter category. In the first place, to refute conclusions based on statistical data one should use statistical methods. Secondly, no one expects every one of his theories to stand up without modification or without, in many cases, eventually being discarded. Nevertheless theories have a very useful function in pointing out directions in which new knowledge should be sought. Theories should be criticized by fellow workers but the authors themselves and their followers should not be held up to ridicule.—Ed.)—*J. B. R.*

Transfusion Reaction Resulting from Anti-Hr″(e) Antibody. Wei-Ping Loh, Methodist Hospital, Gary, Indiana. Am. J. Clin. Path. 35: 111, 1961.

A gravida IV female, who had had one previous transfusion, developed evidences of a moderately severe transfusion reaction during transfusion of three pints of blood at the time of delivery. Anti-e was identified in the recipient's serum. Recheck of the crossmatches with the donor bloods revealed very weak incompatibility by the Coombs technic, but much stronger reactions by the cysteine-papain technic. —*H. A. P.*

The Relationship of the A and H Antigens of Erythrocytes of Sub-Group A_2. A. Richardson Jones and L. Kaneb, John Elliott Blood Bank, Miami, Florida, Vox Sang. 6: 110, 1961.

This is the description of an experiment in which it is shown that the treatment of erythrocytes of sub-group A_2 with anti-A does not interfere with the subsequent sensitization of these erythrocytes with human anti-H. The conclusion drawn from the results is that no detectable steric hindrance can be demonstrated when these two antibodies are simultaneously attached to the same cell and, therefore, the antigen site on the cell which reacts with alpha is spatially distinct from the antigen site which reacts with anti-H.

The authors present, for comparison, the results of previously published experiments which show that steric hindrance apparently does exist between the antigen sites for anti-A and cross-reacting antibody. This paper constitutes a further example of the usefulness of the mixed erythrocyte agglutination reaction for the study of blood group problems.—*A. R. J.*

Multiple Antibodies Imitating the Presence of a Panagglutinin in the Serum of a Patient Suffering from Haemolytic Anaemia. S. Whittingham, R. Jakobowicz and R. T. Simmons. Commonwealth Serum Lab., Melbourne, Australia. M. J. Australia. 1: 205, 1961.

A 15-year-old patient, presumed to have lupus erythematosus, was found to have acute hemolytic anemia. Her serum contained what appeared to be a panagglutinin because it agglutinated all test cells including the patient's own. She had previously received three transfusions whose Rh phenotypes were ascertained. By appropriate absorptions and elutions, her serum was found to contain four antibodies in the Rh system, those directed against c, E, C^w and e (or hr′, rh″, rh^w and hr″) factors. The last factor was contained in the patient's own cells which were typed as *R′R′*. The only blood that was found compatible with that of the patient lacked all of these factors. It was a sample of the rare group *–D–/–D–*. —*J. B. R.*

The Isolation and Properties of a Sialomucopolysaccharide Possessing Blood-Group Le^a Specificity and Virus-Receptor Activity. A Pusztai and W. T. J. Morgan. Biochem. J. 78: 135, 1961.

An unusual polysaccharide has been isolated from ovarian cyst fluid, which has been termed sialomucopolysaccharide. This physico-chemically homogeneous substance possesses two biological activities each associated with a different chemical structure. The molecule is blood-group Le^a specific and also has virus-receptor activity. The material contains 18 per cent sialic acid in addition to fucose, galactose, galactosamine, glucosamine and the amino-acids usually found in blood group mucoids. The sialic acid was readily removed by receptor destroying enzyme from *Vibrio cholerae* with concomitant loss of the influenza virus inhibiting activity, but with no effect on the Le^a activity. On the other hand, Le^a specificity was destroyed by an enzyme preparation from *Tri-*

chomonas foetus but a significant amount of sialic acid and virus inhibitor activity remained with the molecule.

The sialomucopolysaccharide precipitates with anti-pneumococcus XIV horse serum. The extent of precipitation is much enhanced after removal of the sialic acid by receptor destroying enzyme.—*G. F. S.*

Erythroblastosis

A Hitherto Undescribed Phenomenon in ABO Haemolytic Disease of the Newborn. S. Lewi and T. K. Clarke, National Blood Transfusion Centre, Paris, France. Lancet. 1: 456, 1960.

A new test for the detection of hemolytic disease of the newborn due to ABO incompatibility is proposed, based on the sedimentation rate of washed cord red blood cells in a polyvinylpyrrolidone solution. Only eight of 835 ABO incompatible cord cells sedimented rapidly. All were from type A babies with type O mothers, and all had severe clinical hemolytic disease. The cells from 25 babies had a moderately fast sedimentation rate; 17 of these babies had deep jaundice. All others had no more jaundice than the control group. Cord cells from Rh incompatible bloods did not sediment fast.—*H. A. P.*

Anti-M Antibody as a Cause of Intrauterine Death. Report of Two Stillbirths Resulting from Anti-M Antibody. C. R. Macpherson, M. J. Christiansen, W. A. Newton, Jr., W. E. Wheeler and E. R. Zartman, College of Medicine, Ohio State Univ., Columbus, Ohio. Am. J. Clin. Path. 35: 31, 1961.

A mother who was *NN* had two stillbirths, both at five months of gestation. Iron deposition in the liver of the fetuses was consistent with hemolytic disease of the newborn. Incomplete studies of the fetal bloods revealed a positive direct Coombs in one and type MN cells in the other. The immune nature of the mother's anti-M is suggested by its enhancement by AB serum and by Coombs technic and by its high thermal amplitude.—*H. A. P.*

Proteins

Cryoglobulinemia: Rationale of Treatment of a Case Based on Unusual Properties of the Cryoprotein. R. D. Koler, D. A. Rigas, A. J. Seaman, B. Pirofsky and E. L. Swank. Univ. of Oregon, Portland, Oregon. Amer. J. Med. 29: 857, 1960.

A 48-year-old white male with mucous membrane bleeding, visual impairment and mild anemia was found to have a high concentration of a cryoglobulin in his serum; the cryoglobulin polymerized to form a gel which interfered with the clotting mechanisms, increased the blood viscosity, and slowed the peripheral circulation. The cryoglobulin was unusual in that it was stabilized *in vitro* and *in vivo* by basic organic amines such as lysine and arginine. Elegant coagulation studies prior to and subsequent to the removal of the cryoglobulin from the serum indicated that polymerization of the cryoglobulin with fibrinogen, forming a loose gel rather than a normal fibrin clot, accounted for the clinical and laboratory abnormalities in clotting. Additional studies disclosed a long turnover time of the cryoglobulin (half-life 19 days) and immunological reactivity of the protein different from that of normal gamma globulin, despite the presence of some antigenic components common to it and normal gamma globulin. Removal of the abnormal protein by plasmapheresis, resulting in diminution of its concentration from 9.5 to 5 gm. per cent, improved all abnormal clinical and laboratory findings.—*H. F.*

Structural Units of Human 7S Gamma Globulin. E. C. Franklin, N.Y.U. Coll. of Med., New York, New York. J. Clin. Invest. 39: 1933, 1960.

The treatment of 7S gamma globulin with papain, cysteine and EDTA resulted in virtually complete conversion of the starting material into a major fraction of about 3.5S units and small but variable amounts of a heterogenous more rapidly sedimenting fraction of 5S to 6S. Further chromatographic separation on carboxymethyl or diethylaminoethylcellulose resulted in three major separable fractions with a sedimentation constant of about 3.5S and molecular weights of 50,000.

Each of these fractions was antigenically related to the native protein. Two of the fractions (A and C) are closely related to each other but differ in electrophoretic mobility. More than three-fourths of the antibody activity was found in fraction C, with small amounts in fraction A.

The third fraction (B) differed from the others electrophoretically and antigenically. Most of the carbohydrate was associated with this fraction. There was no antibody activity in this fraction.

The role of these structural units is not only of theoretic interest but may prove useful in neutralizing noxious antigens without producing tissue damage caused by the interaction of antigen with tissue fixed antibody.—*A. S.*

Effects of Long-Term Storage on Human Serum Albumin. I. Chromatographic and Ultracentrifugal Aspects. J. S. Finlayson, R. T. Suchinsky and A. L. Dayton. National Institutes of Health, Bethesda, Maryland. J. Clin. Invest. 39: 1837, 1960.

COORDINATED studies by chromatography on DEAE cellulose and subsequent ultracentrifugation were carried out on commercial human albumin preparations stored under various conditions for at least five years. Albumins prepared from citrated whole plasma and stored at 5 C. for five years showed only minimal differences from fresh albumin samples. However, those stored for ten years, those stored at 32 C. or those prepared from ultraviolet-irradiated dried plasma demonstrated more marked differences from the first sample. These latter materials contained components with sedimentation coefficients greater than 5.9S, the amounts of which increased with increasing change of the chromatogram from the normal. —*A. S.*

Immunology

Humoral Factors in the Pathogenesis of Chronic Pancytopenias and Their Relation to Hypersplenism. B. J. Koszewski. Creighton Univ. Sch. of Med., Omaha, Neb. Acta Haemat. 24: 251, 1960.

NINE patients with chronic pancytopenia were studied for the presence of presently accepted etiologic and immunologic factors and followed closely for a period of three to five years. Except for shortened Cr^{51} red cell survival time, no immunologic factors could be detected by the usual laboratory technics. Five of the group showed circulating cyto-depressant substances when whole blood (5 ml. per kg. of recipient body weight) was transfused into blood group compatible normal human volunteers. Three of these five patients have been splenectomized with uniform failure. The four patients without cytopenic factors showed permanent improvement following splenectomy.—*A. S.*

Studies of an Allergic Antibody Active in the Presence of Paraaminosalicylic Acid Against Human Erythrocytes, Leukocytes and Platelets. J. Dausset and Y. Bergerot-Blondel. Centre National de Transfusion Sanguine, Paris, France. Vox Sang. 6: 91, 1961.

A patient with pulmonary tuberculosis received two courses of intravenous paraaminosalicylic acid (PAS) injections. Towards the end of each course the injections were accompanied by fever and fainting. Studies on his serum revealed an antibody that, in the presence of PAS, agglutinated red cells, white cells and platelets. The antibody inhibited clot retraction and was thermostable. Compounds with chemical structure similar to PAS could replace PAS in the *in vitro* agglutination tests. The authors believe that hemolytic reactions in other patients treated with PAS were not due to this antibody but were caused by another mechanism.—*J. B. R.*

Difference Between Gamma-Globulins Fixed on the Platelets of Patients with Idiopathic Thrombocytopenic Purpura and of Those Suffering from Disseminated Lupus Erythematosus or a Panhaematocytopenia. J. Dausset, J. Colombani and M. Colin. Centre National de Transfusion Sanguine, Paris XVe, France. Nature. 188: 865, 1960.

Two different batches of anti-human serum were used in Coombs consumption tests with platelets from the groups of patients listed in the title. With one of the serums, results were negative only with platelets from ITP patients. With the second serum, positive results occurred with platelets from all three disease categories. (It would be of interest to know whether the antigens used in preparation of the Coombs serums were obtained and prepared in identical fashions.—Ed.)—*H. F.*

Antiglobulin-Positive Hemolytic Anemias. E. E. Osgood. Univ. of Oregon Med. Sch., Portland, Oregon. Arch. Int. Med. 107: 313, 1961.

IN this series of 78 patients who developed hemolytic anemia, the majority occurred as a complication of leukemia. Although the Coombs or bromelin tests were usually positive, in some cases the tests were negative. The cases however appeared to be related and are lumped together as "antiglobulin-positive."

The most helpful early diagnostic clues were a rapid drop in hemoglobin (without hemorrhage) and the presence of autoagglutinins in a moist coverslip preparation. When the disease occurred in the course of leukemia, the prognosis for the primary disease became much graver. The principal therapeutic agent was the long-term administration of corticoids.

The author suggests that the positive antiglobulin test is due to an abnormal protein produced usually by lymphocytes, that happens to coat erythrocytes in much the same way that phytoagglutinins do. This has nothing to do

with an antigen-antibody reaction. The term "auto-immune hemolytic anemia" should be replaced by the term used in the title.—*J. B. R.*

"Acquired Agammaglobulinemia" with Auto-Immune Hemolytic Disease: Graft-Versus-Host Reaction? H. Fudenberg and A. Solomon. Rockefeller Institute, New York City, New York. Vox Sang. 6: 68, 1961.

Two patients with severe acquired hypogammaglobulinemia and Coombs-positive auto-immune hemolytic anemia are reported. In at least the first patient, symptoms of hypogammaglobulinemia antedated the hemolytic anemia by many years. Lymphoma was eventually found in this patient and has not been excluded in the second patient. The clinical courses and laboratory studies are evaluated in light of Burnet's hypotheses. The authors conclude that the cells of the lymphoma, arising as a result of somatic mutation and hence antigenically deficient in relation to the cells of the host, may produce antibodies against host tissues to which they are exposed, namely the circulating erythrocytes. Hence the antibodies attached to the host red cells are formed by the gamma globulin produced by the mutant tissue rather than by the patient's normal tissue. In this respect the hemolytic anemia in these patients would resemble that produced in "homologous disease" noted in animal studies of transplantation immunity. Not cited is the possibility that normal levels of gamma globulin (i.e. of antibody) may aid in eleminating mutant cells when they arise; perhaps agammaglobulinemia favors development of such mutant "grafts."—*H. F.*

Anaphylactic Reactions in the Skin of the Guinea Pig with High and Low Molecular Weight Antibodies and Gamma Globulins. Z. Ovary, H. Fudenberg and H. G. Kunkel, New York Univ. School of Med., New York City, New York. J. Exper. Med. 112: 953, 1960.

THE technic of passive cutaneous anaphylaxis (PCA) consists of an intradermal injection of an antibody solution into guinea pig skin, followed, after a suitable latent period, by an intravenous injection of the corresponding antigen mixed with a dye. The authors found that 7S anti-B iso-agglutinins were highly effective in eliciting PCA reactions, whereas 19S anti-B iso-agglutinins failed to do so. Heterophile antibodies from the sera of patients with infectious mononucleosis, demonstrated to be entirely of the 19S type, gave negative reactions, while 7S heterophile antibodies from a guinea pig gave positive reactions following intravenous injection of the antigen (sheep cells). Failure of 19S antibodies to elicit PCA reactions appears to be due to their inability to be fixed to guinea pig tissues.—*H. F.*

Miscellaneous

Leukocyte Antibodies Following Single Massive Transfusion of Living Leukocytes at Cardiac Surgery. R. L. Walford, R. E. Anderson and P. Doyle. Univ. of Calif. Sch. of Med., Los Angeles, California. Vox Sang. 6: 80, 1961.

PREVIOUS studies on leukocyte agglutinins have related their presence to multiple stimuli from banked blood transfusions and/or pregnancy. In this study young patients operated on for congenital heart disease using a heart-lung bypass machine were each given a massive single transfusion of fresh blood. When their serums were tested postoperatively for leukocyte agglutinins, one out of ten subjects showed a moderately strong agglutinin; in four out of ten, the evidence for an agglutinin was considered equivocal.—*J. B. R.*

Outdated Red Blood Cells from the Blood Bank, for Determination of Antistreptolysin O Titers. Y. E. Crawford, R. P. Skinner, W. J. Lind, A. E. Heimann, D. E. Hutchings and W. T. Stille. U. S. Naval Training Center, Great Lakes, Illinois. Amer. J. Clin. Path. 35: 279, 1961.

IN a carefully controlled series of titrations, the authors compared antistreptolysin O titers using as antigen red blood cells derived from the rabbit, as in conventional technics, and from outdated human blood donations. The results were comparable, and the authors conclude that the latter source may be substituted for the former when the former is difficult to obtain.—*J. B. R.*

A Bacteriologic Survey of a Blood Bank. M. Nadeau and N. G. Maxwell, Milwaukee Blood Center, Milwaukee, Wisconsin. Am. J. Clin. Path. 35: 36, 1961.

THE results of 8,943 cultures of blood and blood collection equipment are reported. The authors conclude that the donors' skin and circulation did not furnish significant numbers of bacteria to contaminate the final product if the arm was prepared in a carefully controlled manner. The per cent of positive cultures was correlated with procedures that permitted the entry of non-sterile air.—*H. A. P.*

Legal Problems of Blood Transfusion. Emanuel Hayt. Hospitals. 34: 57, 1960.

THE author has reviewed the legal problems which plague blood banks and transfusion services from the standpoint of hospital liability. Furnishing blood for a transfusion should be regarded as a service rather than a sale. This may offer some protection against legal action for breach of implied warranty of fitness for use. It is the hospital's responsibility to maintain adequately trained personnel for administration, processing and crossmatching. Other sources of liability are discussed, such as transmission of viral hepatitis, patient identification, crossmatching, injuries to blood donors, racial labeling of blood in certain states and administration of blood to patients who object to receiving blood on the basis of religious beliefs.

—L. R. G.

Questions and Answers

A Reaction Problem

Q. "A 27-year-old hemophiliac who had had many prior transfusions without incident was admitted for extraction of teeth. Persistent bleeding on the third, fourth and fifth postoperative days was countered by whole blood transfusions in addition to fresh frozen plasma. Bleeding was thus controlled but jaundice and hemoglobinuria appeared with onset on the seventh day, 48 hours after the last bottle of whole blood. The hemoglobin level dropped from about 12 G. per cent to 9.7 G. per cent during the next six days, slowly returning to reach 12 G. per cent in a further seven days with clearing of jaundice.

The patient is Group A, Rh (D) positive DCcee. On the second day of the hemolytic episode a specimen of his blood was submitted to a reference laboratory. No atypical antibodies were demonstrable by the antiglobulin technic but there was reaction with all cells, including his own, after treatment with an enzyme. At low temperatures the patient's serum would react with his own cells.

In case antibody had been completely adsorbed during the hemolytic reaction, a second specimen was submitted 14 days later. No atypical antibodies were found. Enzyme-treated cells, including those of the patient, again reacted, but tests at low temperatures now were negative.

Retesting of the patient's serum against the donors used demonstrated no error in grouping and no apparent incompatibility by the antiglobulin technic."—*M.D., Montreal, Canada.*

A. "Since this patient was noted to have hemoglobinuria, his differential diagnosis should include all of the causes of acute intravascular hemolysis. One must assume that there was no history of previous episodes of hemoglobinuria or unexplained anemia, and that there had been no recent viral infection or exposure to a potentially hemolytic drug. Furthermore, since the onset of the hemolytic episode apparently occurred 48 hours after the last transfusion, it is unlikely that it was caused by the presence of potent antibodies in a donor's plasma or the activation (by complement or properdin) of some intrinsic hemolytic process in the patient. Even if these assumptions are accepted, one can only tentatively discard such diagnoses as paroxysmal cold hemoglobinuria, paroxysmal nocturnal hemoglobinuria, drug sensitivity (including G.6P.D. deficiency), and acquired hemolytic anemia of the warm or cold antibody types.

One is handicapped by the absence of information concerning the number of transfusions given, the results of the direct antiglobulin test, the titer of the apparent autoagglutinin and the details of the serologic technics employed. For example, if the patient received many pints of blood, "his own" cells would simply refer to the cell population which was not destroyed. Furthermore, if varying dilutions of antiglobulin serum were employed, and if tests for acid hemolysins, Donath-Landsteiner antibodies or (subsequently) for P. N. H. in the patient were carried out, these were not mentioned.

If one then makes the rather large assumption that the patient had no intrinsic red cell metabolic disorder or auto-immune disease, it is necessary to speculate that some blood group antigen present in at least one (and probably two or more) of the transfused units stimulated

an anamnestic antibody response, resulting in the intravascular destruction of red cells containing the corresponding antigen.

In the author's experience, such anamnestically stimulated antibodies usually cause extravascular lysis, even if the antigen-antibody complex fixes complement. However, two recent reports[4, 6] describe delayed transfusion reactions which may have been associated with hemoglobinemia. In both cases, renal failure developed.

Failure to demonstrate a specific isoantibody might be ascribed to one of three causes: (1) The red cells were coated with an antibody which requires the presence of complement for agglutination by antiglobulin serum. The patient's serum, depleted of complement by the hemolytic reaction, was probably not reinforced with fresh serum prior to serologic testing. Alternatively, the patient's serum may have become anticomplementary. (2) Only one dilution of antiglobulin serum was employed, so that optimal concentration of the appropriate antiglobulin antibodies (*i.e.*, anti-7S or anti-19S gamma globulin, "anticomplement," etc.) was not achieved. (3) The antibodies responsible for the hemolytic episode cannot be demonstrated by any of the known serologic technics. Such antibodies are known to exist.[1, 2, 3, 5] That they may be capable of inducing intravascular hemolysis after restimulation is not beyond reason, although this type of reaction must be quite rare.

The author is aware of three other cases similar to the one described by Dr. Harris. After intensive investigation, they were assigned to the uncertain realm of speculation, from which they have not been salvaged."—*Eloise R. Giblett, M.D.*

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"Therapeutics"

Q. "May blood from therapeutic phlebotomies be indiscriminately used for transfusion? If not, what are the criteria for the use of such blood?"—*M.D., Manchester, Connecticut.*

A. "The current standards for establishments licensed under the Public Health Service Act state that blood from therapeutic bleedings can be issued provided the label conspicuously indicates the donor's disease. This allows the patient's physician, the person most qualified, to judge whether or not this blood will be safe for a particular recipient.

Although aside from the infectious diseases, I know of no other disease transmissible by blood transfusion. I also know of but few situations in which blood from a therapeutic phlebotomy offers any advantage over blood from a healthy donor. In the absence of any specific indication for the use of such blood it would be prudent not to use it indiscriminately. Certainly, one criterion that should be applied is that the expected result of such transfusion be at least as good as that which would follow transfusion of blood from a normal donor. In any case it would be wise to look into any non-Federal statutes which might apply locally."—*Paul J. Schmidt, M.D.*

"Universal Donor" Transfusion

Q. "If a patient of group A or B receives group O blood in a large quantity because his own type is not available, and in the meantime his own type becomes available, is it advisable to continue with group O blood or may group A or B whatever his type *be* transfused?"—*Montreal, Canada.*

A. "It is best to follow a rule found useful in military practice—after the transfusion of four units of "universal donor" blood to the adult (scaled down proportionately for children) continue with this until the immediate transfusion needs are satisfied. Once the emergency has been met, it is advisable to follow the patient closely to be prepared to either continue with group O blood or to revert to his group if *new* transfusion requirements should arise. A fairly accurate picture of events can be obtained from serial hemoglobin and

bilirubin determinations and gross grading of differential agglutination and direct antiglobulin tests. Most valuable in making this decision are titrations of any free blood group antibodies incompatible with the patient's own group. In the presence of these, any developing anemia which requires transfusion should be treated with packed, or preferably once-washed, group O red cells."—*Paul J. Schmidt, M.D.*

Crossmatch for Heart Surgery

Q. "Often blood serum for candidates for open-heart surgery is procured at time of cardiac catheterization. Sometimes the serum is stored in frozen state until the operation is scheduled. What is the maximum length of time that such sera may be frozen and still be used for crossmatch purposes?"—*Ph.D., Richmond, Virginia.*

A. "The freezing of serum for the major crossmatch for cases of open-heart surgery is probably not to be recommended sinces changes in complement and antibody strength will occur. In the current issue of TRANSFUSION, a paper from Mollison's laboratories discusses changes in complement during freeze-storage and ways of overcoming the deterioration of complement that occurs during the frozen state.

Some antibodies may also lose strength during storage, as for example, anti-Kidd. Another hazard in freeze-storage of serum may occur if the patient is transfused after cardiac catheterization but before cardiac surgery. There seems little to be gained since the patient must enter the hospital ultimately for heart surgery at which time a fresh specimen of blood representing the patient's current antibodies can be secured."—*Mark Falcon Lesses, M.D.*

Refrigerator Controls

Q. "We are planning to install an alarm and constant recording system in the near future. We would like to know if you will recommend to us the model that you consider the most satisfactory."—*M.D., Montgomery, Alabama.*

A. "No definite model can be recommended to you, although there are several available. The requisites of an alarm system should be as follows:

There should be an alarm system which will set off an alarm indicating deviations below 1 C. and above 6 C. On the high side, the alarm should certainly become activated at 10 C. and on the low side, should become activated at 0 C.

The alarm must be of such a nature, preferably a combination of buzzer and light, that it cannot be totally shut off until the abnormal temperature has been corrected. Ordinarily this can be effected by having a manual control to shut off a buzzer or bell, but a light warning of abnormal conditions should be so protected that it cannot be shut off until correction of the abnormal condition.

The alarm should be battery operated unless the blood bank refrigerator and the alarm system is serviced from several power stations so that failure of the primary power station will not inactivate both refrigerator and alarm system. If a battery-operated alarm is utilized, a testing button should be installed in such a way as to allow daily testing of the adequacy of the battery. This type of high-low alarm system can be installed by any competent electrician. The end of the electrode running from the inside of the refrigerator to the alarm should be installed in such a way as to be sensitive, but not too sensitive, and should be in air, not in liquid. Positioning and insulation of the electrode should permit proper activation of the alarm.

There are many available recording devices. These should probably record the approximate temperature of the blood so that the electrodes should be placed in a container of water at a point in the refrigerator representative of the average temperature. Thus, an alarm system which is set off by warning changes in air temperature and a recording system which shows the actual temperature of the blood, appears a most logical combination for a blood bank refrigerator."—*Mark Falcon Lesses, M.D.*

Correspondence

To the Editor:

Bags for Blood, Saline, and Other Liquids

A FEW MONTHS ago an announcement was sent to all hospitals in the New York City Metropolitan area that after July 1, 1961 blood for transfusion will be provided only in plastic bags. It seems surprising that there has been no notice in print of the contemplated action, and that such a radical step has not aroused any

apparent reaction. The thought of handling liquids and especially blood in bags is displeasing to most individuals, and while many complaints have been voiced privately, the expected storm of protests has not appeared in print. Glass containers for blood are thoroughly tried, and experience with hundreds of millions of transfusions has proved such containers to be eminently satisfactory. No valid scientific evidence has been adduced to justify a change to plastic bags. The claim that blood stored in such plastic containers has a longer survival in the recipient's circulation, has been proved to be only a myth.

The convenience of handling liquids in rigid containers is a matter of everyone's daily experience. No one would consider drinking milk, water, or whiskey from bags. Rigid containers, most often but not necessarily made of glass, have proved to be the most satisfactory means for handling and storing liquids. For blood, bottles have proved most convenient; they will stand upright without outside support on any flat surface, they can be graduated and the volume of fluid they contain ascertained at a glance, and any necessary gummed labels can be easily, rapidly, and securely affixed to glass bottles.

Recently, an item was published describing one circumstance where bags as containers for liquids may actually be superior. It seems that when astronauts at zero gravity attempt to drink from an ordinary glass, the liquid hits their face or clothes instead of entering the mouth. Fluids in a bag, on the other hand, can be expressed directly into the mouth and swallowed. However, so long as one has no immediate plans for space travel, one still prefers all one's liquids including blood in rigid containers, as most earth-bound transfusionists will agree. Blood banks were organized in order to render a service, and where transfusionists find it more convenient to handle blood in bottles, blood banks are obligated to satisfy this demand. Of course, there is nothing to prevent blood banks from providing blood in bags also for those who prefer such containers.—A. S. WIENER, M.D., February 10, 1961.

March 10, 1961

The experience of a number of investigators has conclusively shown that plastic bags as containers for the storage of whole blood provide a better preservation of red cells. There is also some evidence that, at least for human blood, the non-wettable properties of plastic bags favor survival of platelets for short periods of time.

The technic of handling plastic bags has been solved in many ways and the lightweight and lesser space which they occupy offer definite advantages. While it is very true that no one would consider drinking milk, water or whiskey from bags under ordinary conditions, it is equally true that blood is ordinarily not drunk but transfused.

The separation of formed elements and removal under sterile conditions is readily accomplished.

I am not aware of any storm of protests voiced, either privately or in print; indeed, the announcement that in the future plastic bags would be adopted by the Red Cross has been received with much satisfaction and favorable comments from all people interested in making blood transfusion an effective and safe procedure.—MAX M. STRUMIA, M.D.

News and Announcements

Frozen Blood for Transfusion

June 17, 1960 the AABB Reference Laboratories Program received the official permit from the U. S. Naval Hospital, Chelsea, Massachusetts to use the facilities of their Blood Research Laboratory for frozen storage of rare type donor blood.

It was decided to use the limited facilities for extremely rare type blood and a request for these bloods was published in the AABB Bulletin, November, 1960. The following bloods have been received and frozen in the Blood Research Laboratory of the Chelsea Naval Hospital:

13 Cellano—negative
4 Lu^b—negative
4 U—negative
1 Kp^b—negative
1 Vel—negative
2 Cde/C
4 "Bombay"
1 Tj^a—negative

AABB Workshop held in Long Beach, California, March 28, 1961.

The result of the first nine months' operation of this program is very encouraging. However, it should be mentioned that the total of 30 units which have been frozen up to date have come from the following very small number of institutions:

10 units from Mt. Sinai Medical Research Foundation, Chicago, Ill.
5 units from Blood Grouping Laboratory, Boston, Mass.
5 units from Blood Bank, Massachusetts General Hospital, Boston, Mass.
3 units from Minneapolis War Memorial Blood Bank, Minneapolis, Minn.
3 units from St. Vincent's Hospital, Worcester, Mass.
1 unit from Chelsea Naval Hospital, Chelsea, Mass.
1 unit from Knickerbocker Blood Bank, New York, N. Y.
1 unit from Woman's Hospital, Detroit, Mich.
1 unit from Milwaukee Blood Center, Milwaukee, Wisc.

We need cooperation from additional institutions. If you have rare type donors available for the AABB program please obtain information concerning shipping of the blood, etc., from the undersigned.

Morten Grove-Rasmussen, M.D.
Director, Blood Bank
Massachusetts General Hospital
Chairman of the AABB Reference Laboratories Committee

AABB Blood Bank Workshops

The American Association of Blood Banks through its Committee on Workshops, Chairman, J. J. Griffitts, M.D., Co-chairman, Shirley Busch, BB Sp. (ASCP), conducts laboratory classes in blood banking technics.

A workshop was held March 28-29 in the laboratories of the Long Beach State College under the direction of Dr. E. R. Jennings, Chairman of the Subcommittee on Workshops for the Pacific District, with the assistance of Miss Dorothy Day of the Irwin Memorial Blood Bank. Individual and institutional members of the Association, resident in California, were invited to nominate technologists to participate. The class, which was quickly oversubscribed, was limited to 30 participants. Miss Corinne Hindmarsh did the bulk of the work of organizing

the program. Members of the faculty were Drs. A. Richardson Jones, E. R. Jennings, P. R. Thompson, Miss Hindmarsh, Jane Gruenhagen, M.T. (ASCP) BB, Margaret Thompson, M.T. (ASCP) BB and Marjorie Sharp, M.T. (ASCP) BB. The accompanying picture shows the classroom in action.

Certification in Blood Banking

"It is the purpose of the American Association of Blood Banks to expand the opportunities for training and to raise the level of training and experience offered to trainees. To accomplish these goals, the Committee on Education (Chairman, Frank R. Ellis, M.D.) finds it necessary to establish criteria for the approval of centers for training in blood banking and to provide methods whereby such centers shall be regularly reviewed for continuing approval."*

Dr. Edward C. McGarry, Chairman, Subcommittee on Training, announces that approval as training centers in blood banking has been granted to the following institutions:

University Hospital and Hillman Clinic
The University of Alabama Medical Center
Birmingham 3, Alabama
Stephen D. Palmer, M.D., Director

Jacksonville Blood Bank, Inc.
536 West 10th Street
Jacksonville, Florida
John B. Ross, M.D., Director

Wayne State University College of Medicine
1401 Rivard Street
Detroit 7, Michigan
Lawrence Berman, M.D., Director

Irwin Memorial Blood Bank
270 Masonic Avenue
San Francisco 18, California
Mrs. Bernice M. Hemphill, Managing Director

University of Virginia Hospital
Charlottesville, Virginia
O. B. Bobbitt, M.D., Director

St. Joseph Hospital
Linwood Blvd. at Prospect
Kansas City 28, Missouri
Russell W. Kerr, M.D., Director

Wayne County General Hospital
Eloise, Michigan
Frank R. Ellis, M.D., Director

* Quotation from Section II of "Requirements for Certification in Blood Banking," prepared by Committee on Education, American Association of Blood Banks with cooperation of The Registry of Medical Technologists of the American Society of Clinical Pathologists. This pamphlet may be obtained from the Central Office of the AABB.

National Research Council Criteria for Blood Transfusion

THE DEVELOPMENT of blood transfusion has been confronted with three major problems: the procurement of donors, the complexities of the technological aspects, and the rationale of clinical applications.

The procurement of donors has been taken care of in a great variety of ways by hospital blood banks, local lay organizations, community blood banks, commercial or semi-commercial enterprises and by the American Red Cross. While a completely satisfactory liaison between the various interested groups has not been achieved, great progress has been made.

The technological aspects have reached a high level of development, especially in the complex field of immunohematology; the safety of blood transfusion has received a great deal of attention from many sources. The Division of Biologics Standards of the National Institutes of Health has issued regulations which control the quality of the product, whole blood and derivatives. The regulations have been directed specifically for the institutions which are licensed by the Federal Agency for the purpose of interstate traffic of blood and blood derivatives. As a result of the combined efforts of the Scientific Committee of the Joint Blood Council, Inc. and the Standards Committee of the AABB, "Standards for a Blood Transfusion Service" have been published which contain the recommendations of competent workers in the field to assure, as much as possible, a safe transfusion.

There remains the last area which may be defined as the link between the scientific and technological aspects of the transfusion service

and the clinical applications. Although innumerable publications have appeared in periodicals and books concerning the indications for transfusion in various disease conditions, it is considered that insufficient results have been obtained from the efforts to relate the blood banking and clinical aspects of transfusion service. As a result, there is inadequate communication between blood banks and clinical services which is hindering the optimum use of available resources of blood and blood derivatives.

The Subcommittee on Transfusion Problems of the National Research Council has undertaken the task of preparing, with the collaboration of a large number of competent workers in the field, criteria for the express purpose of filling the gap between the blood bank and the bedside.

As yet, the plan is in its initial stages; it is hoped that when completed, it may bring about a better understanding of the complexities of the word "transfusion" and of the absolute necessity for better communication between the medical personnel of the blood bank and the clinician requesting the transfusion.—MAX M. STRUMIA, M.D., *Chairman,* Subcommittee on Transfusion Problems, National Research Council.

State News

West Virginia—At an organizational meeting in Parkersburg on May 7, the new West Virginia Association of Blood Banks was formed with an initial membership of ten institutions and 23 individuals. Bylaws were adopted and a general program for the ensuing year was outlined. The latter will include a discussion session to be held in conjunction with the November meeting of the West Virginia Association of Pathologists, and will have as guest speaker Dr. James J. Griffitts of Miami.

Officers of the newly-formed West Virginia Association of Blood Banks are: president, Robert A. McDougal, M.D., Parkersburg; president-elect, Charles W. Thacker, M.D., Parkersburg; and secretary-treasurer, Harold Klein, Beckley.

Alabama—The new Alabama State Association of Blood Banks, organized November 12, 1960, for the stated purpose of education and improvement of blood banking within Alabama, has announced its tentative plans to pattern the association after similar organizations in other states and to carry out three primary functions: (1) to sponsor workshops annually; (2) to sponsor teams composed of a pathologist, a hospital administrator and a medical technologist to make periodic visits to member banks for the purpose of observing methods and facilities, and to offer assistance with blood bank problems; and (3) to make bi-monthly distribution of unknowns to member banks as an outside aid in controlling materials and technics.

Joseph Beaird, M.D. will direct the seminar organization. Distribution of unknown bloods will be under the supervision of Miss Katherine Melonis, M.T. (ASCP).

Officers of the Alabama Association of Blood Banks are: president, Albert E. Casey, M.D., Birmingham; president-elect, J. D. Bush, M.D., Gadsden; vice president, S. D. Palmer, M.D., Birmingham; and secretary-treasurer, T. F. Wilkes, M.T. (ASCP), Birmingham.

AABB Annual Meeting Forecast

The annual meeting of the American Association of Blood Banks, sometimes called the world's finest forum on blood banking, will convene for its fourteenth session on October 25-28 at the Drake Hotel, Chicago, Illinois. As in previous years, there will be sectional meetings for administrative, scientific personnel, as well as joint sessions for presentation of topics of common interest. Participating in these programs will be many experts in all phases of the transfusion field, a number of whom will be making their first appearance behind the AABB meeting podium.

Overseas guests this year will be Dr. R. R. A. Coombs, Cambridge, Dr. Fred Stratton, Manchester, and Dr. John F. Loutit, Harwell, Didcot.

"Basic Blood Group Serology" is the topic of an excellent basic science seminar to be presented October 25 as a preconvention feature for technologists. An added feature, details to be announced, will provide opportunity for practical workshop assistance with laboratory problems.

The need to keep abreast of scientific advances in blood banking and of economic problems in the health field, has greatly increased the sphere and urgency for research in blood bank administration. The October administrative forum will initiate investigation into the more apparent current problem areas.

A tentative program for the meeting will be published in the September-October number of TRANSFUSION.

A Special Annual Meeting Invitation to All Catholic Sisters

The Committee on Local Arrangements is making special provision for the entertainment

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News and Announcements

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of all Catholic Sisters attending the AABB Fourteenth Annual Meeting. Following the seminar on October 25, the Sisters will tour the AABB Regional Reference Laboratory at Mount Sinai Blood Center, after which they will be guests at a buffet supper and movie. On Thursday afternoon, October 26, they will be guests for luncheon and a tour of a local pharmaceutical house, a visit to St. Mary's Foreign Mission Museum, and dinner at a renowned Evanston restaurant.

These sponsored events have been arranged by Sister M. Teresia, Holy Cross Hospital, Chicago, a member of the Committee on Local Arrangements. The AABB extends a very special invitation to all Catholic Sisters interested in blood banking to attend this year's meeting. A retiring room for their daytime convenience and comfort is being provided through the courtesy of the headquarters hotel.